IMMUNOBIOLOGY OF PANCREATIC ISLET AND THYROID TRANSPLANTATION

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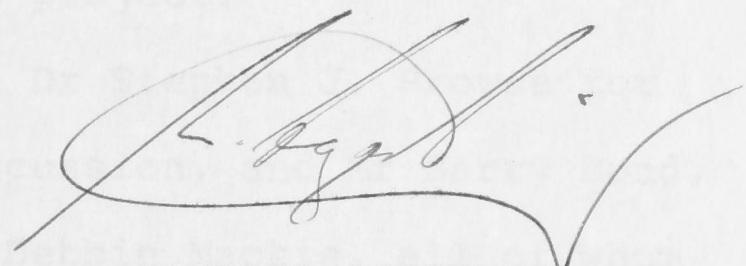
STATEMENT

The experiments described in Chapter 3 were carried out in collaboration with Dr S.J. Prowse, and those in Chapter 4 with Dr Prowse and Dr H.S. Warren. Mr P. Hodgkin was responsible for assessing cytotoxic activity in the experiments described in Chapter 5. With these exceptions, the experiments reported in this thesis represent my own work.



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ABSTRACT

Culture of mouse pancreatic islet and thyroid tissue in an oxygen-rich atmosphere before transplantation, facilitates long-term allograft survival without the use of immunosuppression. The work presented here, represents the results of investigations into the susceptibility to rejection of such cultured allografts. These established allografts are resistant to rejection by alloantiserum and complement - a resistance which we propose is due to the revascularization of the graft with host vascular endothelium. Such revascularization, however, does not protect the allograft from cell mediated damage. The cellular requirements for rejection of cultured islet allografts were studied and the results obtained show that sensitised Thy 1^+, Lyt 1^+2^+ lymphocytes and not Lyt 1^+2^- lymphocytes trigger acute graft rejection. Evidence is also presented to show that lymphokine release by these cytotoxic effector cells plays an important role in triggering allograft rejection.

The susceptibility of grafts to rejection slowly diminishes with time but, until such time as the recipients develop functional tolerance, these grafts remain in a metastable phase. Treatment of islet allograft recipients with UV irradiated spleen cells of donor origin, results in the stabilization of islet allografts by the induction of specific tolerance. The mechanism of this form of tolerance appears to be due to suppression, by an unknown mechanism, of the process of recipient sensitisation.

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PREFACE

This thesis is made up of seven chapters. The introductory chapter (Chapter 1) is a broad review of the extensive literature on allograft rejection and transplantation tolerance, with some emphasis on mechanisms involved in graft rejection and tolerance induction and maintenance. The work described in subsequent chapters of this thesis was an exploration of the susceptibility or resistance of established islets to rejection and the induction of transplantation tolerance.

The experiments were performed using the mouse pancreatic islet and thyroid allograft model with H-2 incompatible donors and recipients. Both islet and thyroid tissues were treated by organ culture before transplantation. Such culture reduces tissue immunogenicity but does not destroy tissue antigens. Thus, while cultured allografts can be successfully transplanted without a need for suppression of the recipient's immune system, such allografts are constantly under the threat of rejection. This is of particular significance in the clinical transplant situation which might apply these procedures to reverse insulin dependent diabetes. A blood transfusion, for example, could trigger rejection if the transfused blood carried histocompatibility antigens similar to those in the graft. These potential threats to the established allograft were examined in the studies that follow.

Chapter 3 examines the resistance of established islet allografts to rejection by antibody and complement. Chapter 4 examines the cellular requirements for the rejection of cultured islet allografts, and Chapter 5 extends our investigations into the mode of function of these cells to reject the islet allograft. Chapter 6 examines the stabilization of established islet allografts and development of tolerance, and discusses mechanisms which might be involved in the development of tolerance. Chapter 2 describes the materials and methods employed in this study and Chapter 7 is a general discussion of the findings of this study.

ABBREVIATIONS AND SYMBOLS

ALS	Antilymphocyte serum
ATXBM	Adult thymectomized, lethally irradiated and bone marrow reconstituted
BSA	Bovine serum albumin
CAM	Chorioallentoic membrane
CML	Cell mediated lympholysis
ConA	Concanavalin A
CoS	Costimulator
c.p.m.	counts per minute
C.U.	Cytotoxic Unit
CyA	Cyclosporine (=Cyclosporin A)
DNase	Deoxyribonuclease
DTH	Delayed type hypersensitivity
EMEM	Eagle's Minimal Essential Medium
g	relative centrifugal force
gm	gram
GVH	Graft versus host
HBSS	Hanks Balanced Salt Solution
HIFCS	Heat inactivated foetal calf serum
$^{125}_{I}$	^{125}Iodine
Ia	I region associated
IL1	Interleukin 1
IL2	Interleukin 2
LNC	Lymph node cells
2-ME	2-Mercaptoethanol
MHC	Major histocompatibility complex
MLC	Mixed lymphocyte culture
mmol/l	millimoles per litre

PBS	Phosphate buffered saline
PC	Peritoneal cells
PMN	Polymorphonuclear
SC	Spleen cells
SD	Standard deviation
TLI	Total lymphoid irradiation
UV	Ultraviolet
µCi	microcurie
v/w	unit volume per unit weight
WBI	Whole body irradiation

CHAPTER 1

REVIEW: ALLOGRAFT REJECTION AND TRANSPLANTATION TOLERANCE

1.1 GENERAL INTRODUCTION

Reports of blood, skin and organ transplantation are rooted in antiquity. Some are no more than legends, with the feat of Saints Cosmos and Damian, who flourished in the third century AD, being among the best known. The story is told of a complaining leg amputee who was restored by these two medical saints when they successfully transplanted the leg of a Moor who had recently died. Nonetheless, there are also many documented cases of transplantation which go back many centuries. These include skin grafts to restore mutilations to the nose, ear and lip, and blood transfusions from young persons to older ones aimed at rejuvenation. For an historical account, see Saunders (1972). In the nineteenth century, numerous skin allografts were carried out giving rise to many claims of success. This was probably a result of the initial "take" before its rejection. Failures were considered by many to be a result of technical difficulties.

It was with the turn of this century, however, that the scientific basis for transplantation really began and many technical advances were also made at this time. Nonetheless, such advances were not able to overcome the central stumbling block, namely, rejection. Many subsequent experiments soon made it clear that although autografts could survive indefinitely, allografts were rejected within a matter of days.

Unfortunately, the issue of allograft reactivity remained confused and it was not until the investigations by Gorer in the 1930's and Medawar in the 1940's that the immunological nature of the allograft response was demon-

strated. This recognition soon led to a rapid expansion in transplantation studies, with many investigations aimed at elucidating the precise mechanisms of rejection and the induction of tolerance.

We now know that after an animal has had an appropriate contact with antigen it may respond in a number of different ways, including the production or non-production of antibody, the development of delayed type hypersensitivity, or even become unresponsive or tolerant. Which of these events occurs depends on the conditions under which the antigen, whether a suspension of cells or an organ allograft, is delivered. If appropriate conditions are selected, a tolerant state can result. The prevention or reversal of the immunologial processes which lead to allograft rejection is a central problem in organ transplantation and knowledge of the conditions under which different types of immunity can be achieved, is not only of theoretical importance, but it also has important applications to medicine.

Part I of this introductory chapter will review the development of the immunological basis of rejection, its effector mechanisms and lymphocyte activation, with a special look at organ culture as a method of overcoming the barrier or obstacle to allotransplantation. Part II will review the induction, maintenance and mechanisms of transplantation tolerance.

PART I: ALLOGRAFT REJECTION

1.2 INTRODUCTION

With certain exceptions, allografts as a rule closely resemble isografts for a short time in that they are initially accepted by the host. Allografts, however, are soon rejected following this initial "take" and the whole transplant becomes necrotic and may be sloughed off, absorbed or gradually replaced by scar tissue. In man, skin allografts, for example, often survive for two to three weeks and sometimes longer but are ultimately rejected. Exceptions to this inevitable rejection include allografts between identical twins or between animals of a closely inbred strain, allografts of certain tissues such as cartilage, and allografts to certain sites, commonly referred to as immunologically privileged sites, such as the anterior chamber of the eye (Woodruff and Woodruff, 1950) and the hamster's cheek pouch (Billingham and Silvers, 1962) where allograft survival may be indefinite. In addition, there have been a variety of experimental and therapeutic procedures aimed at both the graft and the recipient, which have had varying success in prolonging allograft survival. Two of the best known procedures have been the use of immunosuppression to treat the recipient so that it cannot respond to the stimulus of the graft, and organ culture to treat the graft so that it will be unable to stimulate the host. However, with these exceptions and without specific measures being taken, the normal fate of all allografts is destruction. While various hypotheses have been advanced to explain allograft rejection, the evidence indicates that it is

likely to be a process involving the interaction of many components of the immune system and mediated by both antibody and cellular mechanisms.

1.3 GRAFT REJECTION AS AN IMMUNOLOGICAL PROCESS

1.3.1. Early ideas

One of the earliest ideas concerning allograft rejection and first introduced by Ehrlich (1908) working on tumour transplantation, was that allografts perish as a result of "athrepsia" or the inability of transplanted tissue to obtain an adequate supply of essential substances from the host. This idea, however, has long been abandoned, especially in view of the finding that tissues can be cultured *in vitro*. A number of early investigators, also working on tumour transplantation, suggested that heredity might play a role in determining the fate of transplants. But one of the problems with the early studies was that genetically pure stock were not always used, or indeed available.

Loeb (1930, 1945) postulated that the tissues and organs of a given organism have in common a chemical characteristic, termed the "individuality differential" which is peculiar to the individual. This differential, he saw as being the product of the genes which controlled tissue compatibility, and one of the ways of analysing such differentials was by transplantation experiments (Loeb, 1930). In an allograft situation two main events were seen to occur: the individuality differential of the transplant evokes the production of toxins, and the host tissue then responds to these toxins by a cellular accummulation, including the cellular invasion

of the transplant. The end result is destruction of the transplant (Loeb, 1930). Although Loeb's conclusions have been described as sometimes being vague and inadequate (Woodruff, 1960; Saunders, 1972), he nonetheless formulated the basic idea that transplantability was essentially a search for biological identity.

A number of early attempts were also made in an effort to adapt the graft to the host by first growing the graft in in vitro culture in the body fluids of intended recipients. Stone et al. (1934) reported the successful treatment of two patients with parathyroid tetany by means of an allotransplant which had been cultured for a time in vitro in a medium containing serum and plasma from the recipient. Gaillard (1948, 1953, 1954) carried out grafting of cultured human foetal parathyroid gland tissue in patients suffering from postoperative tetany. Of 30 patients so treated over a period of 10 years, 7 positive results were obtained. However, these results should be viewed with some caution and Gaillard himself stressed that no histological evidence had been obtained to show that the transplants had remained alive. In view of the lack of direct evidence for graft survival in these clinical studies and the growing concept that rejection was due to the host's immune system recognizing genetically fixed foreign antigens on the tissues, such attempts to change the tissue by culture were treated with some scepticism and even outright scorn (Medawar, 1957). As a result, this approach was essentially abandoned until the 1970's (Section 1.7.3).

1.3.2. The immunological basis of rejection

The idea that grafts are destroyed as a result of an immune process was originally put forward by opponents of the athrepsia hypothesis, although it was opposed by Loeb (see Woodruff, 1960). However, it was Gorer (1937) working with mouse tumours, who provided the first clear evidence of an immune response to allografts. He demonstrated that the rejection of a tumour allograft by a host resulted in the formation of antibodies which were capable of agglutinating donor red blood cells. This suggested that the antigenic differences between donor tissue and recipient played a role in rejection.

However, it was not until Medawar (1944), working with skin allografts in rabbits, that the development of active immunity against the foreign tissue or allograft was conclusively demonstrated. He found that when skin is allograted there is a latent period of several days during which the skin graft heals and thrives. This is soon followed by infiltration of host lymphoid cells into the graft, reaching a peak at about day 8, and complete destruction of the graft results by about day 12. This is known as "first set" rejection. He also demonstrated that when a second graft is transplanted to the same recipient, the rejection is now greatly accelerated; rejection is complete within about 6 days. This is a "second set" rejection. This more rapid rejection indicates that the recipient has become sensitised to donor antigens. Moreover, further experiments demonstrated that this sensitisation is donor specific since grafts from another unrelated or third party donor, are rejected in first set manner. These experiments demonstrated two

criteria required to establish that allograft rejection is an immune reaction: specificity and memory.

It was found that animals could become sensitised with other types of tissue from the same donor, thus indicating that the genetically controlled antigenic determinants were present in most body tissues. Medawar (1946) showed that skin allografts could be rejected in second set fashion by prior sensitisation of the host with donor type blood leucocytes. From these findings he inferred that leucocytes and skin expressed common antigens. Other investigators soon reached the same conclusion, using skin, kidneys, spleen and lung (see Woodruff, 1960), that such diverse tissues and organs have transplantation or histocompatibility antigens in common (Section 1.4).

Burnet and Fenner (1949) developed a theoretical model for immune reactivity which was based on the idea that contact with foreign antigen led to the expression of immune responsiveness. Discrimination between self and nonself, or foreign antigens, was learnt during ontogeny. This allowed the prediction that exposure to foreign antigen during embryonic life should enable that individual in later life to be tolerant to that particular antigen. The question of the development of immunological tolerance is discussed in Part II.

While Medawar showed rejection to be mediated immunologically, it was the work of Mitchison and his colleagues that defined the cellular factor in allograft rejection. Mitchison (1953, 1954) showed that immunity to tumour allografts could be transferred to other mice by the transfer of lymph nodes draining the site of the graft. This passive transfer of immunity also satisfied the

third criterion to identify the allograft reaction as an immunological event. Animals receiving immune lymph nodes gave a second set response to a tumour allograft, a lymphosarcoma, which was given at the same time. Because sensitisation could not easily be transferred by serum, this argued for the presence of a predominantly cellular immune response to the tissue antigen.

Various workers have since implicated either the antibody or the cellular response as being of predominant importance in transplantation. But it is now clear that an either/or situation does not exist and that both cellular and humoral factors are involved in the rejection process. Their relative importance depends on various factors such as the animal model studied, the type of allograft, and modification of the host's immune system by immunosuppression (Section 1.5).

1.3.3 The classical view of allograft rejection

With the establishment that allograft rejection was an immunological reaction, the antigens carried on tissues were soon regarded as being the barrier to successful transplantation. Medawar's interpretation was that once the graft healed into place and a lymphatic drainage had been established, the graft antigens were transported to the regional lymph nodes where they activated a response leading to the destruction of the allograft (Medawar, 1956-57). This explanation embodied the assumption that antigenic recognition was sufficient to lead to lymphocyte activation and subsequent graft destruction (Medawar, 1963).

In its favour was the fact that it appeared to offer

an easy explanation, not only for the demonstration that blood leucocytes could immunize against a skin allograft, suggesting that both expressed common antigens (Medawar, 1946), but also the induction of acquired tolerance to skin allografts by early exposure to donor specific antigens (Section 1.10). Medawar's group provided further support for this concept by showing that crude, cell-free extracts of spleen, thymus and lymph node tissues could also provoke the rejection of skin allografts in second set fashion (Billingham et al. 1958), suggesting that the different tissues all had the same antigenic makeup, although it was thought that the antigens were confined to nucleated cells (Medawar, 1956-1957).

The phenomenon of immunological enhancement (Section 1.13) was a contradiction to this view since the pretreatment of hosts with various preparations of tissues of donor origin gave a result opposite to that expected, namely, the prolongation of tumour (Snell, 1952; Day et al. 1954) and skin (Billingham, et al. 1956b) allografts. Nonetheless, Medawar's explanation for the transplantation barrier was generally accepted and became the standard or classical view of graft rejection, remaining virtually unchallenged until the early 1970's (Section 1.6).

The strategy of clinical transplantation which developed during this period is indicative of the wide acceptance of this view. Since transplantation antigens alone were seen as the major impediment to grafting and, since the antigenic makeup of a tissue is built into its

genetic constitution, it was considered "infirm" by Medawar (1957) to attempt to change this makeup by using such methods as organ culture. Consequently, the only approach it seemed was to modify the recipient in some way, such as by immunosuppression. Procedures for tissue typing were also developed in an attempt to match both donor and recipient. In spite of increasingly sophisticated approaches to tissue typing, however, rejection still remains the major cause of clinical transplant failure. Tissue matching has been very difficult to achieve because of the complexity of the transplantation antigens which are coded for by a group of genes called the major histocompatibility complex (MHC) and because of the large number of possible MHC phenotypes.

1.4 THE MAJOR HISTOCOMPATIBILITY COMPLEX.

It has now long been recognized that the fate of an allograft is determined by the genetic relationship between donor and host. The recipient's immune system recognizes certain molecules on the surface of grafted cells as being foreign and, in due course, attacks and destroys them. These molecules, known as histocompatibility (H) antigens are found on nearly all cells and are coded for by a set of H genes. In general, grafts exchanged between animals that do not differ in the H genes are accepted; those between animals differing in the H genes, that is, histoincompatible, are rejected.

In the mouse, the histocompatibility antigens are coded for by a series of genes found on chromosome 17 and denoted as H-2. Similar complexes of genes have been studied in other species, including man, referred to as HLA (Bach and Van Rood, 1976), and rat and chicken (Klein,

1979). The generic name for these complexes is the major histocompatibility complex (MHC).

The existence of the MHC reflects the fact that there are other (minor) histocompatibility complexes which are less well defined. The minor or non-H-2 antigens are controlled by genes which are scattered throughout the mouse genome. The intensity of an allograft response to individual non H-2 antigens is generally less than that seen when there are differences at the H-2 level, although cummulative non H-2 immunogenicity may somtimes equal that of the H-2 complex (Graff, 1978). However, the normal functions of non H-2 antigens are thought to be non-immunological. This contrasts with the function of H-2 antigens which are primarily involved in non-self recognition processes (Klein et al. 1981).

The MHC of the mouse has been the most extensively investigated, particularly by Klein and coworkers, and a number of reviews have appeared in recent years (Klein, 1975; Bach et al. 1976; Munro and Bright, 1976; Silver et al. 1976; Klein, 1979; Klein et al. 1978, 1981; Hood et al. 1983; Sachs, 1983). Because there are so many aspects of MHC research, a brief summary only is presented in this section. It should also be noted that from the data available the mouse MHC appears to be a general model for those of other mammals, including man.

The H-2 complex is divided into three main regions: K, I and D. The I region is further divided into five subregions: A, B, J, E and C. H-2 loci can be grouped into two classes according to their phenotypic expressions. Class I, which comprises the K and D regions, code for

membrane-bound glycoproteins with a molecular weight of 45,000 daltons. Each molecule consists of a single polypeptide chain noncovalently associated with a shorter polypeptide called β_2-microglobulin. It has a molecular weight of 12,000 daltons and is encoded for by a locus situated on chromosome 2 (Klein et al. 1981). Class I antigens are expressed on almost all cells of the animal and close homology exists in the primary structure of these products (Sachs, 1983).

Class II antigens are those coded for by the I region. These are membrane-bound glycoproteins consisting of two noncovalently bound polypeptide chains, the α and β with a molecular weight of 35,000 and 28,000 daltons respectively (Klein et al. 1981). The products of this region are referred to as Ia (I region associated) antigens. Class II antigens have a more restricted distribution, being expressed predominantly on B cells and on some macrophages (Hammerling, 1976; Sachs, 1983) although the evidence is growing that these also exist on non-lymphoid organs such as skin, lung and liver (reviewed by Nixon et al. 1982).

Both the class I and class II loci are highly polymorphic and each display a large range of alleles. For example, 56 alleles have been found at the H-2K locus and 45 at the H-2D locus in the house mouse (Klein, 1979). The allelic composition of the H-2 complex is referred to as the haplotype and is designated by an alphabetical superscript, for example, $H\text{-}2^k$.

Although early interest in these histocompatibility antigens stemmed from their role in graft rejection, this is obviously not their normal biological function. It is

now known that these antigens play an important role in the body's defence system against its own cells infected with viruses or turned cancerous. The work of Zinkernagel and Doherty (Zinkernagel and Doherty, 1974a,b; Doherty and Zinkernagel, 1975) established that cytotoxic T cells made in one inbred strain of mice would kill virus infected cells from that strain only. Thus, for example, an effector lymphocyte sensitised against vaccinia virus infected H-2^a cells, destroys vaccinia infected H-2^a cells but not, for example, vaccinia infected H-2^b cells. They found that this "restriction" on killing, a term generally applied to the limiting effects of MHC on the immune response to antigen, was controlled by class I H-2 genes. The sensitised T cells recognise a complex of foreign (viral) and self (H-2) antigen, although whether this recognition occurs via a single receptor or two receptors (one for the viral antigens and the other for the H-2 molecule) is still a matter of debate. H-2 restriction has also been described for T helper cells which, in order to help B cells, are first activated by antigen on the presenting cell, such as a macrophage, and then recognise the H-2 molecules on the presenting cell. In contrast to cytotoxic T cells, however, the molecules recognised by helper cells are controlled by class II genes, that is, loci on the I region (Klein et al. 1981). The MHC molecules, therefore, serve as structures which are recognised in conjunction with antigen by T cells. Thus, a major biological role for the MHC is to signal changes in self to the immune system.

The H-2 has now been shown to control a number of

other functions such as collaboration between T and B cells, the reactivity of mixed lymphocyte reaction and, in particular, controls the activation of different subclasses of T lymphocytes. These subclasses are characterized by the expression of T cell specific Lyt antigens - Lyt 1, Lyt 2 and Lyt 3 (Cantor and Boyse, 1975, 1976). T cells activated against class I antigens carry the Lyt 1^+ 2^+ 3^+ phenotype and those responsible to class II antigens express only the Lyt 1^+ 2^- 3^- antigen. Contrary to early reports, all T cells have now been shown to carry some Lyt 1 (Ledbetter et al. 1980; Nakayama, 1982). The Lyt molecules of the mouse have been particularly of great importance in defining functionally distinct T cell subsets (reviewed by Swain and Dutton, 1980; see Chapter 4). The variation in immune response between some mouse strains, such as the ability to produce different levels of antibodies to a given antigen, is controlled by immune response (Ir) genes, many of which also map in the I region of the H-2 complex (Klein et al. 1981). The question of how Ir genes may operate is discussed by Blanden (1980).

In an attempt to simplify the "traditional" view of the maze of regions and subregions, Klein and his colleagues (Klein, 1981; Klein et al. 1981) have recently proposed a simpler interpretation of the H-2 complex. They suggested, firstly, that a single H-2 locus controls a variety of traits, that is, loci are pleiomorphic and, secondly, that the basic function of the H-2 complex is to guide T cells in their role of distinguishing self from nonself. H-2 controlled traits, such as rejection of allografts, are artifacts derived from this basic

function. These suggestions led them to propose a simpler nomenclature for the MHC - a proposal which has also been supported by Mitchison (1980, 1981).

1.5 EFFECTOR MECHANISMS IN GRAFT REJECTION

With the establishment that the rejection of allografts is an immunological reaction, numerous studies were soon undertaken with the aim of elucidating the mechanisms of rejection. By the late 1950's, however, the debate on the respective roles of cellular and humoral mechanisms had reached the general consensus that the latter played little, if any, role. While humoral antibodies were seen as important in the rejection of an allograft composed of dispersed cells (Stetson, 1963), solid tissue grafts were destroyed by cell-mediated mechanisms (Mitchison, 1954; Snell, 1957; Brent, 1958). This view persisted into the early 1960's when a number of observations which had led to this interpretation were then being challenged.

It now seems quite unlikely that allograft rejection is brought about solely by cell mediated or humoral mechanisms. Both play a role and the relative contribution of each of these components depends on such factors as the animal model, the type of tissue used for grafting and whether the response induced is a primary or secondary response.

1.5.1. Role of antibody

The idea that humoral immunity was of little importance in allograft rejection, was based largely on experiments which demonstrated a failure in general to:

(i) induce allograft rejection by passive serum

transfer - a failure which persisted even when the serum dosage, timing and route of injection were varied (Billingham et al. 1954; Billingham and Brent 1956; Stetson, 1963),

(ii) induce rejection of various endocrine tissues, tumours and split skin allografts placed in diffusion chambers - a failure which persisted even when hosts were sensitised against the donor tissues (Algire et al. 1954, 1957; Woodruff, 1957; Brooks et al. 1960),

(iii) passively transfer skin allograft immunity by sensitised cells confined within diffusion chambers (Billingham et al. 1963; Wilson et al. 1966), and

(iv) demonstrate haemagglutinating and cytotoxic antibodies in skin allografts (Brent, 1958; Woodruff, 1960).

However, by the early 1960's, a number of reports emerged which showed that under certain circumstances, humoral antibodies could play an important part in the rejection of solid tissue grafting. In earlier work, aside from some technical difficulties, the skin graft model was probably not the best model to demonstrate the effect of antibody in rejection because the graft is isolated from circulation prior to revascularization and the antibody may not reach the graft (Najarian and Foker, 1972). Also, subsequent work with millipore chambers indicated that, although antibody and complement penetrated the chamber with difficulty, enclosed tissue could in fact be destroyed by antibody when their level was

appropriate (Algire, 1959; Amos and Wakefield, 1959).

A number of reports also emerged which showed that serum transfer caused accelerated allograft rejection. Kretschmer and Perez-Tamayo (1961) demonstrated that the rejection of skin allografts in rabbits was significantly accelerated by the local administration of serum obtained from immunized animals, and Dubernard et al. (1968) induced the rejection of an isologous kidney in dogs by the transfusion of immune serum. There is now a large body of evidence, both from human and animal transplantation studies, using skin, kidneys, heart and other tissues and organs, for the involvement of antibody in allograft rejection.

1.5.1.1. Hyperacute rejection of human kidney allografts

Convincing evidence for the role of antibody has been obtained with the demonstration of hyperacute rejection of kidney allografts in human and animal studies. Kissmeyer-Nielsen et al. (1966) were the first to show hyperacute rejection of human kidney allografts in two patients from a series of 21 consecutive transplants. This rejection was attributed to circulating antibodies in the serum of recipients which were reactive against donor antigens - both patients had undergone blood transfusions before transplantation. The pathological findings in the two patients showed cortical necrosis with extensive glomerular thrombosis. Moreover, the absence of cellular infiltration was interpreted as indicating a positive effect by humoral antibodies.

Williams et al. (1968) in a similar human study, found that this form of rejection is very rapid, occurring

within hours or even minutes of removing the clamps from the blood vessels. Its characteristic feature is the accummulation of large numbers of polymorphonuclear (PMN) leucocytes in the graft vasculature. Where rejecting grafts were left *in situ*, progressive fibrin deposition, thrombosis and widespread cortical necrosis occurred. They were also able to detect antibodies in eluates from renal allograft tissue.

This very rapid onset of the reaction, the elution of cytotoxic and agglutinating antibodies from graft renal tissue (Williams *et al*. 1968; Pedersen and Morris, 1974) and the lack of mononuclear cell infiltration into the grafts, argued against the involvement of cell mediated immunity in hyperacute rejection. It should also be noted that antibodies in the form of circulating immune complexes may also play a role, especially in late deterioration of renal grafts (Milgrom, 1977). These and other investigations have led various workers (Williams *et al*. 1968; Winn *et al*. 1973; Hume, 1974; Bogman *et al*. 1980) to argue that hyperacute rejection is a result of the interaction of antigraft antibodies with the endothelium of the graft vasculature (Chapter 3).

1.5.1.2. Hyperacute rejection in animal studies

Hyperacute rejection studies have since been extended to animal models using the passive transfer of antiserum. Such rejection has been demonstrated with kidney allografts in rats (French, 1972; Hart *et al*. 1980; Winearls *et al*. 1980), rabbits (McDowall *et al*. 1973) and sheep (Pedersen and Morris, 1974); skin allografts in mice (Koene *et al*. 1973; Gerlag *et al*. 1975, 1980;

McKenzie and Henning, 1978), and rats (Jooste and Winn, 1975); and with xenografts of kidney (Chavez-Peon, et al. 1971), heart (Burdick et al. 1979), skin (Jooste et al. 1973, 1981a, b; Winn et al. 1973; Jooste and Winn, 1975; Gerlag et al. 1980; Berden et al. 1981) and pancreatic islets (Frangipane et al. 1977a,b; Naji et al. 1979a,b). That antibodies can play an important role in the destruction of grafts is clearly illustrated in these studies. However, these studies have also shown that the sensitivity of grafts to rejection can vary enormously depending on the interval of time between transplantation and the injection of antisera, its dosage and the type of allograft.

Unlike kidney allografts, freshly transplanted skin is resistant to anti-donor alloantibody. Gerlag et al. (1975) showed that mice could reject skin allografts only when injected with antiserum and rabbit complement from day 4 post transplantation, with the greatest sensitivity to rejection on days 7 and 8. With rat skin transplanted to mice or allografted to rats, antisera injected during the first week after grafting have no detectable effect on survival times, whereas sera given at about 2 weeks after transplantation are particularly effective (Jooste et al. 1973, 1981a; Jooste and Winn, 1975; Gerlag et al. 1980). These studies found that while grafts are initially resistant to damage, they rapidly develop sensitivity and after a short peak at about two weeks, this sensitivity is gradually lost over a period of several weeks. Skin grafts surviving beyond 35 to 40 days become resistant to antisera (Jooste and Winn, 1975).

The initial resistance of skin grafts was explained

by the time taken for the establishment of an adequate vascular network between graft and host (Jooste et al. 1973; Gerlag et al. 1975; Jooste and Winn, 1975). This is consistent with reports that primary healing and vascularization of skin grafts is completed by day 4 after transplantation (Medawar, 1944; Jooste et al. 1981a). However, as Jooste et al. (1981a) point out, this could not be the whole explanation as it would explain the resistance only for the first 4 days after transplantation, whereas sensitivity to rejection is delayed for a further 2 to 3 days. They postulated that this early period of resistance is due to the immature properties of the young, regenerating vessels in the graft. This explanation for the early insensitivity of skin to rejection is also consistent with observations that primary vascularized grafts such as kidneys and heart, in which there is little or no formation of new vessels, are highly sensitive to humoral antibodies immediately following transplantation (French, 1972; Burdick et al. 1979; Jooste et al. 1981a).

The suggestion which emerges from these studies then, is that the antibody mediated attack is targeted on the vascular endothelium of donor organs and tissues. This would explain the changes in sensitivity with time seen in skin allografts. Following the initial establishment of a vascular connection between the host and the graft, the skin tissue rapidly becomes sensitive to antibody mediated rejection but, as its vascular endothelium is replaced by donor cells, the graft gradually loses its sensitivity. Conversely, the uniform sensitivity of heart allografts, was explained on the basis that

extensive endothelium replacement does not occur (Burdick et al. 1979). More recent reports have provided good evidence for this proposal of endothelium replacement and is discussed in Chapter 3.

Several studies have also suggested that the mechanism of hyperacute rejection is dose dependent. While studies, particularly in the rat model, have shown a requirement for a heterologous source of complement (Section 1.5.1.3), Fabre and Morris (1974) found that hyperacute rejection can occur in the DA to Lewis strain combination in the absence of this additional complement, provided a large enough dose of antiserum is given at the time of transplantation. Similarly, McDowall et al. (1973) suggested that antiserum dosage is likely to be a significant factor since, depending on dose, either hyperacute rejection or enhancement can be produced in rabbits carrying kidney allografts. A relatively high dose (40 to 60ml) of antidonor alloantibody led to hyperacute rejection, whereas lower doses either had no effect or caused graft enhancement.

1.5.1.3. Complement dependence

The complement system has also been shown to play an important role in hyperacute rejection. French (1972) demonstrated that hyperacute rejection could be produced in AS rats carrying August kidney allografts, when recipients were given anti-August alloantiserum and guinea pig serum as an additional source of complement. Alloantibody in the absence of guinea pig serum, failed to produce hyperacute rejection - a failure attributed to a defective complement pathway or inadequate fixation of native complement to the graft (French, 1972; Morris, 1980).

The complement activity of guinea pig serum was essential to the production of hyperacute rejection, since this activity was lost by heating the serum to 56°C for 30 mins (French, 1972). More recently, Hart et al. (1980) and Winearls et al. (1980) reported similar findings in the DA to Lewis rat strain combination. Without an additional source of complement, therefore, rats appear to be peculiarly resistant to this form of rejection. Incidentally, this resistance to antibody damage has probably been a major reason why the rat has proved such a good model to produce immunological enhancement (Section 1.13).

This complement dependence has also been demonstrated in hyperacute rejection of skin allografts in mice (Koene et al. 1973; McKenzie and Henning, 1978). Interestingly, guinea pig complement was ineffective in hyperacute rejection of mouse skin allografts whereas rabbit complement was effective, thereby illustrating that the source of complement is an important factor (Koene et al. 1973). Koene and McKenzie (1973) showed that there is an in vitro difference in cytolytic efficiency between the sources of complement and proposed that this difference was due to guinea pig complement requiring the activation of 2 adjacent cell surface sites for cytolysis, whereas rabbit complement requires only one site.

The prevention of the activation or modification in some way of the complement system, can prevent or considerably delay hyperacute rejection. Winn et al. (1973) found that hyperacute rejection of rat skin grafted onto mice was inhibited if the recipients were treated with chicken-anti-rat serum (a non-complement-fixing anti-donor antiserum), or with F (ab')2 antibody fragments.

Pretreatment with cobra serum factor which depletes circulating complement levels, had the same inhibatory effect and rejection was also delayed in mice with a genetic deficient C5 complement component. They suggested, however, a secondary, though essential role for complement. This role lies in its ability to produce chemotactic factors which may attract the polymorphs. This suggestion was based on their evidence that, despite normal levels of complement, there was an absence of graft damage when PMN leucocytes were reduced by pretreatment of recipients with nitrogen mustard or anti-PMN serum. More recently, Berden et al. (1981), also using skin xenografts in mice, showed that although complement activation is important in graft destruction, it is not essential. The inhibatory effect of cobra venum factor treatment and C5 deficiency can be overcome by raising the mouse anti-rat serum dose. Moreover, rejection could also be elicited by the non-complement fixing IgG subclass.

Donor specific antibody may contribute to graft rejection by the process of antibody dependent cell mediated cytotoxicity which does not require complement fixation. This phenomenon reflects the cytotoxic ability of a subpopulation of cells with Fc receptors against antibody (most commonly IgG) coated targets (MacLennan, 1972; Section 1.5.2.2).

1.5.2. Cellular rejection mechanisms

As already indicated, the notion of the cellular basis of allograft rejection came from the early studies of Mitchison (1953, 1954, 1955) who showed that immunity to tumour allografts could be transferred by lymph nodes draining the site of the graft. That is, accelerated

rejection of a tumour allograft could be transferred to a naive animal by cells from these nodes. Billingham et al. (1954) used a similar experiment in an analysis of the transfer of immunity to skin allografts and they too found that immunity can be passively, or to use their phrase, "adoptively" transferred, thereby confirming Mitchison's observations. Further evidence for the concept of the cellular basis of allograft rejection came from studies which showed that mice made neonatally tolerant and now carrying skin allografts of the tolerated genotype, would reject these grafts if injected with normal lymphoid cells syngeneic with the recipient. An accelerated rejection occurred if the lymphoid cells came from a sensitised animal (Billingham et al. 1963). Gowans et al. (1963) used a similar experimental system to show that rats rendered tolerant by neonatal inoculation of F_1 hybrid marrow cells and carrying long-standing skin grafts, will have these grafts rejected soon after the injection of syngeneic thoracic duct cells.

1.5.2.1. Role of T cells

While rejection is a process involving the interaction of many components of the immune system, there is good evidence that the rejection process can be triggered by sensitised T lymphocytes. The evidence for the role of T lymphocytes and requirement for thymus function in graft rejection, comes from several sources. For example, it has been shown that neonatal thymectomy or the congenital absence of the thymus results in poor graft rejection. Miller (1961) demonstrated that skin allograft rejection is severely impaired in neonatally thymectomized mice.

Reconstitution of the thymus with a foetal thymus of similar histocompatibility, restored the recipient's capacity to reject skin allografts. Kindred (1971) and Wortis (1971) showed that skin allografts on nude mice remained intact for the period of the survival of the recipients, which varied between 4 and 10 weeks after grafting. Mice homozygous for the mutation nude lack a thymus (Pantelouris, 1968) and are deficient in lymphocytes in the thymus dependent areas of the spleen and lymph nodes (de Sousa et al. 1969), as well as having a marked depletion of cells bearing the cell surface antigen theta (θ) found on thymocytes and thymus dependent cells (Raff and Wortis, 1970). Manning et al. (1973) extended these observations by showing that nude mice accepted for their lifetime a wide range of skin xenografts, including human, bird, reptile and amphibian. The importance of the thymus was again established by showing that thymus implantation enabled nude mice to reject such xenografts.

Workers have also purified T lymphocytes, either by virtue of surface markers like Thy 1 in the mouse (Reif and Allen, 1963; Raff and Wortis, 1970) or by the ability of human T cells to bind to sheep red blood cells (Jondal et al. 1972) and have shown that these cells are necessary for the development of cytotoxic lymphocytes.

Recent evidence for the role of T cells has come from the work of Hall and his colleagues who studied the cellular requirements for graft rejection by using the adoptive transfer technique and testing known cell populations for their ability to cause rejection of heart allografts in irradiated rats. They established that Ig^-, recirculating, long-lived T lymphocytes restored the graft rejection

response. Neither B lymphocytes nor antibodies were required for the first set rejection (Hall et al. 1978a). The transfer of cells from sensitised animals showed that memory was carried by long-lived, non-recirculating, Ig^- small lymphocytes (Hall et al. 1978b).

More recent reports from Loveland and his coworkers have presented evidence for the role of particular T cell subsets and these findings are discussed in Chapter 4. Analysis of the particular cells involved in graft rejection is of increasing importance with development of monoclonal antibodies which may be capable of modifying the immune response to prevent the rejection of the graft.

1.5.2.2. Cellular inflammatory response

Acute rejection of allografts is associated with a wide variety of pathological patterns and as already indicated, is thought to be mediated by cellular immune mechanisms - an interpretation supported histologically by the dense, host cellular infiltration into the rejecting graft (Lindquist et al. 1971; Hebertson, 1973). However, although the cell-mediated immune response to alloantigens has been widely studied using cells of the peripheral lymphoid system and analysing their functions in vitro, the immune mechanisms operating within the allograft itself have received relatively less attention - a situation brought about by the difficulty in isolating functionally viable, infiltrating host cells. The histological techniques have been limited in distinguishing the different kinds of mononuclear cells, let alone performing any quantitative evaluation of the different

cell types or subpopulations. In recent years, however, with the development of mechanical disruption and enzymatic methods to recover cell infiltrates, it has been possible to show that the inflammatory response consists of a heterogeneous collection of cell types, including monocytes, macrophages, B and T lymphocytes, null cells and polymorphs (Strom et al. 1977; Tilney et al. 1979a; von Willebrand et al. 1979a; Hayry et al. 1979; Carpenter, 1981). Other methods of cell recovery, such as the sponge matrix allograft model (Roberts and Hayry, 1976) which allows a relatively higher recovery rate of cells, and the multicellular tumour spheroid model (Lord and Nardella, 1980), have been used to quantitatively recover and study the infiltrating cells. These methods have the added advantage in that they avoid proteolytic digestion which would otherwise destroy many surface markers.

The relative accummulation of each cell type within the graft is influenced by such factors as the time after transplantation when the infiltrate is analysed, the type of tissue grafted and even the method of cell recovery (reviewed by Carpenter, 1981). Thus, for example, in rejecting rat heart allografts, the predominant cell type at day 4-6 post transplantation when cellular infiltration has peaked, were T cells (Strom et al. 1977). Tilney et al. (1979a) reported that lymphocytes comprised 75-90% of cell infiltrates recovered from rejected human renal allografts, and lymphocytes were also the major population infiltrating the sponge matrix allograft (Roberts and Hayry, 1977). In contrast, studies using the multicellular spheroid model

have found macrophages to be the predominant infiltrating host cell, whereas lymphocytes were present in low numbers (Lord and Nardella, 1980; Sordat et al. 1980).

Infiltrating cells have been tested for their capacity to lyse ^{51}Cr-labelled target cells in vitro as a way of identifying potential rejection mechanisms and it has been shown that, in addition to T lymphocytes, several other infiltrating cell types have the potential to express effector cell function. Strom et al. (1977) showed that by treating harvested cells from rejecting rat heart allografts with T cell specific antiserum, the predominant killer cell present during the early post transplantation period were T lymphocytes. This was similarly demonstrated in the mouse sponge matrix model (Ascher et al. 1979), although in this model, macrophages and monocytes were also active in target cell lysis (Roberts and Hayry, 1977). In addition, Strom et al. (1977) demonstrated that a separate population of Fc receptor-positive cells and distinct from macrophages, lysed cells coated with anti donor IgG. Therefore, antibody-dependent cell-mediated cytotoxicity has been shown to represent another possible mechanism for allograft rejection. The factors which regulate the non-specific inflammatory response are unknown, although both chematoxis and lymphokine mediation have been suggested (Ascher et al. 1979).

1.6 LYMPHOCYTE ACTIVATION

Once the immunological basis of allograft rejection was demonstrated, it was postulated that grafts were rejected because transplantation antigens from the graft were carried to the recipient's lymph nodes which then led to lymphocyte activation and subsequent graft destruction (Section 1.3.3). This idea was based on the widely held assumption that antigen alone was sufficient to cause lymphocyte activation (Medawar, 1963). From the late 1960's, however, this assumption came under question and considerable evidence has since accummulated which now makes the assumption untenable. In its place, a two-signal model for T cell activation has been developed in which both antigen and lymphokine, a hormone-like molecule produced by lymphoreticular cells, play a co-equal role in T cell activation. Experimental evidence which supports this model comes from early investigations into the graft versus host (GVH) reactions and, more recently, from studies on the mechanisms of T cell activation in vitro. These studies have been extensively reviewed elsewhere (Simonsen, 1962; Lafferty and Jones, 1969; Lafferty, 1980; Lafferty et al. 1980, 1983a) and a summary only is presented in this section.

1.6.1. Insufficiency of antigen alone for lymphocyte activation

Studies of the GVH reaction carried out in the late 1960's renewed interest in the role of lymphocytes in allograft rejection. Elkins and Guttman (1968) suggested an important role for the cells because of the GVH

reaction produced when normal parental Lewis strain rat spleen cells were introduced under the kidney capsule of Lewis kidney freshly grafted into F_1 hybrid hosts. Studies by Lafferty and Jones (1969) into the species specificity of GVH reactions in the chicken embryo, began to question the assumption that antigen alone was sufficient to lead to an allograft rejection. They found that lymphoid cells taken from adult chickens and introduced on the chorioallentoic membrane (CAM) of genetically different chick embryos led to a GVH reaction in the chick embryo - a reaction which normally leads to the death of the embryo or newborn chick, and which occurs because of the chick's immaturity. This type of reaction is one which had already been shown to be an immune response; it is produced only when adult lymphoid cells are placed on the recipient and no response is produced when cells are placed in syngeneic embryos (Simonsen, 1962). Lafferty and Jones (1969), further demonstrated that xenogeneic lymphoid cells from ducks, pigeons or sheep failed to produce the GVH lesions onto CAM of the chicken embryo - a failure which had earlier been observed by other workers (Simonsen, 1957; Payne and Jaffe, 1962). In these earlier studies it was a failure to observe splenomegaly in chicks - another assay for GVH reactions.

Thus, it seemed that contact with antigen did not always lead to a damaging reaction in the host. Conventional ideas on transplantation biology would have predicted that pigeon cells should react even more strongly than allogeneic chicken cells. Failure to do so would have to be due to a failure of the cells to

survive and function in the foreign environment (Simonsen, 1962). This failure to cause a GVH reaction was soon shown, however, not to be due to the cells' inability to survive or react immunologically in the embryo. Sheep lymphocytes reacted positively in the skin of other sheep, and pigeon spleen cells, although they could not react against the chicken host on the CAM, could destroy foreign pigeon embryo bones also transplanted on the CAM (Lafferty and Jones, 1969). Further studies in the chicken embryo showed that these transplantation reactions involved an interaction between foreign lymphocytes and blood cells in the target tissue. Heart muscle from incompatible embryo was not attacked if the blood cells were washed out by perfusion, but if perfused with embryonic spleen cells before transplantation, the graft was rapidly rejected. Similarly, the destruction of bone tissue could be blocked by inactivating the blood forming cells of the marrow by gamma irradiation (Lafferty and Jones, 1969). Work in other species also indicated that GVH reactions depend on the interaction of lymphocytes with foreign haematogenous cells (Elkins, 1971).

The conclusion drawn from these studies was that antigen alone was not a sufficient requirement, and that activation of the allograft response required both antigen recognition and the passage of a specific inductive molecule between the interacting cells (Lafferty *et al*. 1972).

A similar conclusion indicating that T cell activation is a 2 signal process was arrived at from studies of T cell activation *in vitro*. The *in vivo* response to a

tissue allograft is much more complex than in the *in vitro* reaction because of the greater range of potential cellular interactions. Nonetheless, the mixed lymphocyte culture (MLC) and cell mediated lympholysis (CML) assays have been widely used and it is commonly accepted that the *in vitro* lysis of allograft target cells by primed cytotoxic cells is a good model for studying the rejection of allografts (Bach *et al*. 1976). Using such techniques, several important observations have been made:

(i) Only metabolically active cells are capable of stimulating T cells. Cells killed by a variety of procedures, including UV irradiation and treatment with chemical agents, do not stimulate incompatible T cells, even though such cells have been shown to express transplantation antigens (Lafferty and Jones, 1969; Hardy and Ling, 1969; Schellekens and Eijsvoogel, 1970; Lindahl-Kiessling and Safwenberg, 1971; Wagner *et al*. 1973; Lafferty *et al*. 1974). An additional factor to antigen has been postulated (Lafferty and Jones, 1969; Schellekens and Eijsvoogel, 1970; Lindahl-Kiesling and Safwenberg, 1971; Batchelor *et al*. 1978).

(ii) Only certain viable cells are capable of stimulation. Nonlymphoid cells such as erythrocytes (Hardy and Ling, 1969), fibroblasts, PMN leucocytes (Greineder and Rosenthal, 1975), epithelial tumours (Talmage *et al*. 1977), and platelets (Lindahl-Kiessling and Safwenberg, 1971), do not stimulate allogeneic lymphocytes

even though they can be shown to express MHC antigens.

(iii) The capacity to stimulate is restricted phylogenetically. The response to xenogeneic leucocytes is either poor or non-existent in spite of the much greater antigenic disparity (Lafferty and Jones, 1969; Wilson and Nowell, 1970; Woolnough et al. 1979).

Clearly then, these studies all indicated that antigen recognition alone was not sufficient for T cell activation by allogeneic cells; something else was also required. Attempts to explain the requirements for this activation have led to the development of 2 basic models.

1.6.2. Models for T cell activation

The two models which have been developed for T cell activation are both based on the Bretscher and Cohn (1970) suggestion that two signals are required for lymphocyte activation. The basic difference between the two models is their perceived origin of the second signal.

(i) The Lafferty model. This was proposed by Lafferty and his coworkers (Lafferty and Cunningham, 1975; Lafferty and Talmage, 1976; Lafferty et al. 1978), although support for the requirement of 2 signals was independently proposed by other investigators (Lindahl-Kiessling and Safwenberg, 1971; Batchelor et al. 1978). Simply stated, lymphocyte activation was seen as a two signal process in which the stimulating cell, and one which presents foreign antigen, plays a unique role in regulating this response. The model postulated that the stimulator cell, designated S^+, was required for the presentation of antigen to the

potentially responsive T cell (signal 1). The S^+ cell simultaneously also provides an inductive molecule which expresses costimulator (CoS) activity (signal 2). Together, signal 1 and signal 2 lead to activation of the T lymphocyte; neither signal alone is sufficient for T cell activation (Lafferty and Cunningham, 1975; Lafferty and Woolnough, 1977).

Experimentally, viable cells of the lymphocyte-macrophage class were shown to express the S^+ phenotype (Greineder and Rosenthal, 1975; Lafferty and Cunningham, 1975; Talmage et al. 1977). Non-lymphoid and metabolically inactive S^+ cells, were unable to stimulate. These were designated S^- cells. The inability of S^- cells to stimulate, however, was shown to be capable of being rectified by adding to the cell cultures a lymphokine containing supernatant obtained from concanavalin A (ConA) activated spleen cells (Talmage et al. 1977; Lafferty et al. 1978; Shaw et al. 1980). This was clearly a demonstration for the existence of a lymphocyte costimulator or signal 2. The presence of the lymphokine-containing supernatant by itself was not sufficient to activate T cells. For a discussion of the history, nature and function of lymphokines, their production and release, see Andrus et al. (1980), Lafferty et al. (1980), Watson et al. (1980), Waksman (1980) and Watson (1981). In order to account for the phylogenetic specificity of cell reactivity, it was proposed and later shown that CoS was a species-specific, hormone-like molecule or lymphokine (Lafferty and Cunningham, 1975; Lafferty and Woolnough, 1977; Woolnough et al. 1979). That is, although individuals could respond to xeno-

antigens, they normally fail to do so because the S^+ cells presenting these antigens fail to provide an effective or homologous signal 2.

The 2 signal model has since been incorporated into a broader theoretical system to describe the process of clonal selection and expansion. Using a symbolic terminology, the characteristics of the immune system that follow from these postulates can be described (Lafferty et al. 1983a).

(ii) The Bach model. This model originally proposed by Bach and his colleagues (Bach, 1973; Bach et al. 1976, 1977), also postulates 2 signals. Binding of class I antigen on the stimulating cell by the cytotoxic cell converts the latter to a "poised" state. This, however, is not sufficient for activation. To bring it to an activated cytotoxic cell requires a second signal from an activated helper T cell. According to the original model, the helper cell, is itself activated following binding of its receptor to a class II antigen on the surface of the stimulator cell. The help given by the helper to the "poised" T cell, might be delivered either by direct cell-cell contact or by a "collaborating" factor which is secreted from the stimulated helper T cell. This would provide signal 2. Thus, in the Bach model, the stimulator cell has a less direct role in the stimulation of cytotoxic T cells. This model has been supported more recently by Brent et al. (1981) who found that mouse liver parenchyma cells, which were shown to lack class II antigens, failed to cause an accelerated rejection of skin grafts. They postulated that this lack of immunogenicity was "directly attributable" to the absence of class II antigens, that is, these antigens provide the second signal.

As a general explanation for T cell activation, the model has several problems. The helper and cytotoxic T cells are regarded as initially exclusive T cell subsets, but there is now considerable evidence which suggests it is incorrect to make rigid distinctions between the properties of cytotoxic and helper T cells. T cells activated to either class I or class II alloantigens can be shown under appropriate conditions, to express both helper and cytotoxic potential (Section 4.1). Moreover, experimental observations have not supported their proposal that recognition of class II alloantigens activates helper T cells and hence provides the second signal for cytotoxic T cell activation. Lafferty et al. (1978) have shown that the stimulator tumour cells P815 and EL-4, neither of which express detectable class II antigens, can still generate strong cytotoxic T cell responses. That is, cytotoxic T cell activation can occur without a requirement for helper T cells. Cantor and Boyse (1975) have also reported cytotoxic T cell activation without the requirement for the Lyt $1^{+}2^{-}$ helper cell and Bach and Alter (1978) have themselves demonstrated cytotoxic T cells in MLC's where the only difference between the stimulating and responding population was in class I antigens. As a result of these findings, the original Bach model was extended to provide an alternative pathway for cytotoxic cell activation in the presence of only class I antigen differences (Bach and Alter, 1978). Nonetheless, this modification is not compatible with the evidence that UV inactivated cells which carry class II antigens, are non immunogenic (Lafferty et al. 1974; Chapter 6). The second signal is CoS activity

which can be provided by lymphokine.

In both models the stimulator cell, either actively or less directly, is of critical importance. Because such cells on the graft are positive for class II antigens it follows that such class II positive cells will make up an important immunogenic component of the graft. However, although there is a strong correlation between class II antigen positivity and expression of S^+ phenotype, exceptions do occur and reveal that recognition of class II antigen itself cannot provide the source of the second signal required for cytotoxic T cell activation. That is not to say that class II positive cells are unimportant in the T cell activation process. For example, inactivation of these cells with antisera and complement removes the cells' stimulating capacity (Yamashita and Shevach, 1977). However, not all class II positive cells stimulate. B lymphocytes were not able to reject rat islet (Zitron et al. 1981a) or rat kidney (Lechler and Batchelor, 1982) allografts. On the other hand, some class II negative cells, such as P815, also stimulate. Thus, a distinction needs to be clearly drawn. The importance of the stimulator cell does not lie in its possession of class II antigens per se. Rather, such antigens happen to be a marker for the cell that expressses the S^+ phenotype, that is, the cell that can provide a source of CoS activity or signal 2. Experimental evidence supports the dendritic cell, a class II antigen-rich cell, as the most likely candidate for the stimulator cell (Hart and Fabre, 1981a,b; Steinman, 1981; Lechler and Batchelor, 1982).

1.7 OVERCOMING THE OBSTACLE TO TRANSPLANTATION

Excluding the rather limited technique of transplanting tissues to privileged sites (reviewed by Barker and Billingham, 1977), theoretically there are really only two basic approaches which may be taken to avoid allograft rejection: treatment of the graft and treatment of the recipient. Both procedures have been widely used, the former moreso experimentally, and each is based on a different perception of the nature of the barrier or obstacle to allotransplantation.

1.7.1. What is the real obstacle to transplantation?

Treatment of the recipient has been by far the most widely used method, both experimentally and clinically, and the reason for this lies in part on the historical and theoretical development of transplantation biology (Section 1.3.3). The ideas which developed at that time stressed that since the obstacle to transplantation was in the antigens of the graft tissue, the only rational means of overcoming the rejection was by tissue typing and host immunosuppression. Many cytotoxic drugs have therefore been used but these have severe side effects because of their non specific effect. Identical tissue matching has in practice been very difficult to achieve. Experimentally, graft survival has also been induced by treating the recipient with anti-donor antibody, subcellular donor antigen such as membrane fragments and donor cells. The effects of these treatments have been varied (see Part II).

From the discussion in Section 1.6 above, it follows that antigen *per se* is not the obstacle to transplanta-

tion. The obstacle is encountered when antigen is presented to recipient T cells on donor stimulator or S^+ cells. Alloantigen carried on graft parenchyma cells will not activate T cells directly because parenchyma cells cannot provide a source of CoS activity. Although free antigen shed from the graft can be presented on recipient stimulator cells to recipient T cells, such indirect presentation could not lead to the activation of the graft specific T cells; these T cells would be specific for graft in association with the MHC of the recipient (Lafferty et al. 1983a). Thus, it is antigen carried on the surface of stimulator cells which themselves are carried as passengers within the graft, that become the real obstacle. It is they which constitute the major source of tissue immunogenicity (Lafferty and Talmage, 1976; Sollinger and Bach, 1976; Lafferty, 1980).

If what is stated above holds, then it should be possible to overcome the obstacle to allotransplantation by treatments which are directed at eliminating or inactivating such passenger cells in the graft tissue - a procedure which is clinically preferable because it eliminates the need for recipient immunosuppression. Experimentally, organ culture (Section 1.7.3) has been shown to eliminate the immunogenicity of tissues, such as thyroid and pancreatic islets, without removing MHC antigens from tissue parenchyma cells.

1.7.2. Passenger leucocytes

This concept originated in a review on the allograft reaction by Snell (1957) who suggested that donor leucocytes carried in the graft might play an important role

in invoking immunity. Subsequently, various workers produced experimental evidence implicating passenger leucocytes in graft immunogenicity. Elkins and Gutmann (1968) made this implication in the rejection of kidneys and suggested that attempts to rid the graft of such cells could be of clinical importance. At about the same time Steinmuller (1967, 1969) showed that skin grafts taken from neonatally tolerized mice would sensitise normal syngeneic animals against subsequent skin grafts from the tolerance inducing strain. This immunity was similar to that induced by skin allografts taken from normal donors. Because the tolerant donors were haemopoietic chimeras, Steinmuller attributed the sensitising capacity of the skin isografts to the passive transfer of leucocytes carried in the vasculature (Steinmuller, 1967). Subsequently he demonstrated the relationship of the immunizing capacity to the number of leucocytes present and the degree of leucocyte chimerism of the donors (Steinmuller, 1969). For a review of the role of passenger leucocytes in the immunogenicity of skin grafts, see Steinmuller (1980).

These early findings established the potential importance of passenger lymphocytes, and considerable attention was soon turned towards methods aimed at eliminating such cells from graft tissue. These methods have included whole body irradiation (Stuart et al. 1971), antilymphocyte serum (Guttman et al. 1967) and the administration of cyclophosphamide and other cytotoxic drugs (Guttmann et al. 1969, 1975). However, although results varied, especially where different

tissues were used, these techniques were generally unsatisfactory. Most did not give a consistent reduction in graft immunogenicity (Billingham, 1971). Moreover, the clinical efficacy of donor pretreatment remains controversial. This lack of success helped to raise some doubts as to the significance of passenger leucocytes in allograft rejection (Billingham, 1971; Stuart et al. 1971).

Stuart et al. (1971) suggested that in renal allografts at least, passenger leucocytes as well as vascular endothelium might provide a strong immunogenic stimulus leading to allograft rejection. However, Lafferty's group (Lafferty et al. 1976b; Talmage et al. 1976) have argued that because as few as 10^3 viable lymphocytes are required in a graft to initiate a host response, it is possible that the techniques they employed (total body irradiation) may have been inadequate to ensure complete elimination of passenger leucocytes.

More recently, Hirschberg and Thorsby (1981) have also challenged the passenger leucocyte concept by proposing that vascular endothelial cells could be an alternative to passenger leucocytes as the stimulating cells. On this basis, the reduction in tissue immunogenicity following in vitro culture of thyroids and pancreatic islets (Section 1.7.3), was seen by them as being due to the degeneration of vascular endothelium (Parr et al. 1980b) rather than the inactivation or elimination of passenger leucocytes. One of their strong lines of evidence has been that isolated vascular endothelial cells, both freshly explanted and after several weeks in culture, can stimulate allogeneic lymphocytes in vitro (Hirschberg et al.

1975), whereas lymphocyte preparations rapidly lose their ability to stimulate in culture (Opelz and Terasaki, 1974; Miller et al. 1975). However, the results of Hart and his group have demonstrated that long-term surviving rat kidney allografts retain donor-type vascular endothelium (Hart et al. 1980; Hart and Fabre, 1981a,b). If endothelial cells had stimulating activity, these long-term allografts should have been rejected. They in fact went on to emphasise that it is the loss of passenger leucocytes, and most likely the dendritic cells (Hart and Fabre, 1981a,b) which most adequately explains long term survival and graft adaptation in their system. Moreover, the finding that cyclosphosphamide pre-treatment of tissue donors can prolong thyroid allograft survival - a situation in which the graft has an intact vascular bed at the time of transplantation - would also argue against endothelium providing the stimulus for the host's immune response (Lafferty et al. 1976b; Lafferty and Woolnough, 1977). It would seem, therefore, that whatever stimulating capacity endothelial cells have, it is at best of secondary importance. The evidence points to the passenger lymphoid cells as being the "professional" stimulator cells (Parr et al. 1980b). Nonetheless, the loss of endothelium during organ culture and subsequent host revascularization following transplantation, may protect the tissue from an antibody mediated attack (see Chapter 3).

1.7.3. In vitro organ culture

The idea that organ culture might reduce tissue immunogenicity is not new and early attempts were made in the 1930's and 1940's to condition graft tissue for clinical

transplantation (Section 1.3.1). For a review of the development of organ culture see Fell (1976) and Hodges (1976). These early studies, however, were criticised both because they lacked genetic control and because they went against the current dogma of the newly discovered immunological basis of graft rejection (Medawar, 1957; Section 1.3.1). As a consequence, it took some years for interest in this work to be rekindled.

In the late 1960's Jacobs and Huseby (1967, 1968) reported the growth of several neoplastic tissues in allogeneic rats and mice following organ culture, and concluded that the culture used had been able to regress or reduce tumour immunogenicity. They later made the further suggestion that the reduced immunogenicity following culture may be due to a depletion of lymphoid cells (Jacobs, 1969). With the reports of Summerlin and his coworkers (Summerlin et al. 1970; Summerlin, 1973; Summerlin et al. 1973), but subsequently not confirmed, wide interest in organ culture was aroused. The potential for clinical application was evident. This group reported the successful allografting of adult human skin following organ culture for 6 weeks (Summerlin et al. 1970). This work and subsequent reports with mouse skin (Summerlin 1973; Summerlin et al. 1973) led to a number of fruitless attempts to confirm their results (Ninnemann and Good, 1974; Lafferty et al. 1975). Nonetheless, although Summerlin's work was soon discredited because of falsification of experimental evidence, the organ culture technique was pursued independently by several groups who subsequently reported that this technique could be used to prolong the

survival of mouse ovarian (Jacobs, 1974; Lueker and Sharpton, 1974) and thyroid (Lafferty *et al*. 1975, 1976a,b) allografts. With these successes the *in vitro* culture technique became firmly established as a tool in allotransplantation.

Extensive use of this technique, particularly in the study of graft rejection mechanisms, has been made in our laboratory. Mouse thyroid has been found an especially suitable tissue for culture and transplantation. Not only is the procedure a relatively simple one, but also its characteristic structure enables easy histological assessment and it can be readily transplanted under the kidney capsule where it quickly becomes revascularized. Using the graft's ability to concentrate radioactive iodine as a measure of graft function, as well as histological examination, Lafferty and coworkers have shown that while uncultured allografts were acutely rejected within 15-20 days, organ culture in 95% O_2 and 5% CO_2 for 12 days enhanced survival, although the degree of prolonged survival depends on the strain combination used (Lafferty *et al*. 1975). By extending the culture period to 3-4 weeks, allografts showed no evidence of rejection over an observation period of several months (Lafferty *et al*. 1976a,b; Talmage *et al*. 1976). Pretreatment of tissue donors with cyclophosphamide (300mg/kg) 4 and 2 days before harvest of thyroid tissue, reduced the period of organ culture required for allograft survival. This treatment, together with a 7-10 day culture period, allowed indefinite survival (>250 days) of thyroid allografts (Lafferty *et al*. 1976b; Lafferty and Woolnough

1977). This effect by cyclophosphamide is probably due to its leucotoxic activity (reviewed by Turk and Parker, 1982). Talmage (1980) has been able to reduce the period of organ culture even further. By using donor treatment with hydrocortisone and hyperbaric oxygen for culture, the organ culture period can be reduced to 1 day.

With the demonstration that organ culture facilitated thyroid allotransplantation to non-immunosuppressed recipients, similar attempts were soon made using pancreatic islets. A strong motivating factor was the potential these techniques offered for clinical application and, in particular, the hope that such methods might be able to reverse juvenile onset diabetes and its many degenerative complications. For a recent and extensive review of islet and pancreas experimental studies as well as clinical trials which includes tissue treatment by organ culture, see Sutherland (1981a,b). A summary only is outlined in this section.

Islet isolation techniques had already been developed before organ culture was conclusively shown to prolong survival (Moskalewski, 1965; Lacy and Kostianovsky, 1967) and the ability of isolated pancreatic islets to synthesize and secrete insulin in tissue culture was soon established (Lacy and Kostianovsky 1967; Andersson and Hellerstrom, 1972; Andersson, 1976). Kedinger et al. (1977) found that in vitro culture of allogeneic islets 4-5 days before transplantation in rats increaased the survival of grafted tissue to >161 days; mean graft survival time with uncultured islets was 56 days.

A number of studies have been undertaken by Lacy's group who initially used a short culture period (1-2 days)

as well as donor pretreatment with irradiation and silica, in conjunction with a single injection of antilymphocyte serum (ALS) given to recipients (Lacy et al. 1979a). Only a moderate prolongation (40 days) of islet allograft survival was achieved. By increasing the length of the in vitro culture to 7 days (24°C) and still maintaining a single injection of ALS for recipients, prolonged survival (>100 days) was obtained (Lacy et al. 1979b,c, 1980a).

This work was extended by transplanting rat islet xenografts to mice which demonstrated prolonged survival of islets and a return to normoglycaemia in diabetic mice (Lacy et al. 1980b,1981). More recently, they were able to reverse diabetes in mice with neonatal rat islets following culture, but without ALS treatment of recipients (Serie et al. 1983).

In our laboratory the culture and transplantation of adult and foetal mouse pancreatic tissue has been extensively investigated using a similar culture system to thyroid, namely, 95% O_2 in 5% CO_2 at 37°C (reviewed by Prowse et al. 1982a). Culture of isolated adult pancreatic islets for 7-12 days enables their long term survival in MHC incompatible recipients without the use of immunosuppression (Bowen et al. 1980). Talmage has shown the importance of high oxygen tension in the gas phase for successful allotransplantation (Talmage and Dart, 1978; Talmage, 1980). This effect may result from the selective toxicity of oxygen for passenger leucocytes. We have been unable to culture single mouse islets because they break down in high oxygen, although

clusters of about 50 islets will fuse and can be cultured in this way - a procedure which also enables greater ease of transplantation under the kidney capsule (Bowen et al. 1980). As few as 300 islets or 6 clusters treated in this way can reverse diabetes in nonimmunosuppressed recipient mice (Bowen and Lafferty, 1980; Bowen et al. 1981). A similar protocol of high oxygen tension and aggregation of islets has recently been shown to reverse diabetes in mice transplanted with rat islets (Lacy et al. 1982). More recently our group has also shown that transplanted mice respond normally to an intragastric challenge with glucose (unpublished data).

The development of juvenile onset diabetes may involve an autoimmune response, perhaps triggered by viral infection (Craighead, 1981), which leads to islet antibodies and insulitis, or an infiltration of mononuclear cells, which ultimately destroy the insulin-producing beta cells (Lernmark and Baekkeskov, 1981; Nerup and Lernmark, 1981). This response, it has been suggested, may also lead to the destruction of the graft islet tissue (Naji et al. 1979a, 1981a,b). However, our group has shown that islets allotransplanted to recipients suffering from insulitis induced by subdiabetogenic doses of streptozotocin did not suffer the same fate, but rather permanently reversed the diabetes in the recipients (Prowse et al. 1982b). Moreover, a CBA mouse found to have spontaneous diabetes, also had its diabetes successfully reversed with a cultured islet transplantation (Prowse et al. 1982b). Thus the cultured allografts did not succumb to the same fate as the original pancreas.

While the success with adult islet tissue is encouraging, technical problems which are associated with the isolation of islets at present prohibit this technique to be used clinically despite recent advances which have vastly improved islet yields (Horaguchi and Merrell, 1981). As a result, much attention has turned to the foetal pancreas as source of islet tissue. However, in comparison with adult islets, foetal pancreas is a much more difficult tissue to condition for successful allotransplantation (Mandel and Higginbotham, 1979; Simeonovic et al. 1980). This increased immunogenicity in foetal pancreas appears to be related to the large amount of lymphoid tissue which is associated with it (Simeonovic et al. 1980). Prolonged culture of human foetal tissue has shown that while this procedure will greatly deplete the immunostimulatory cells such as dendritic cells, they were not abrogated completely. However, while Mandell and Higginbotham (1979) were not able to prevent allograft rejection following prolonged organ culture, in our laboratory it has been shown that it is possible to allograft foetal pancreas successfully to nonimmunosuppressed recipients when a relatively longer period of organ culture (17-20 days) is used, although some islet tissue is lost during this extended culture (Simeonovic et al. 1980; Simeonovic and Lafferty, 1981). This partial loss of tissue was reflected in the fact that tissue from 2 cultured pancreas isografts does not function as well as a single uncultured pancreas in reversing diabetes in syngeneic recipients (Simeonovic and Lafferty, 1981). More

recently, mouse foetal islet precursors, "proislets" have been isolated and when cultured for 4 days in 5% CO_2 in air at 37°C, can be successfully transplanted and reverse diabetes in syngeneic recipients (Simeonovic and Lafferty, 1982a). In contrast to uncultured and 10 day cultured foetal pancreas allografts which are acutely rejected, 20% of mouse proislet allografts were not rejected at 12 weeks following transplantation (Simeonovic and Lafferty, 1982b). This method may therefore become a more suitable alternative for the transplantation of foetal islet tissue. Very recently Archer (1983) maintained neonatal pig islets in monolayer culture for several weeks and these were shown to synthesise DNA and release insulin, suggesting that tissue cultured in this way may be a potential source of material for transplantation.

The differences observed in immunogenicity between adult islets, foetal pancreas and foetal proislets can be attributed to the differences in contamination by passenger leucocytes. By handpicking isolated adult islets it is possible to avoid contamination by small lymph nodes. Foetal pancreas, on the other hand, is contaminated with primitive lymphoid tissue and because it is not organized into discrete structures, cannot be removed by dissection at harvest (Simeonovic et al. 1980). The weak immunogenicity of foetal proislets probably results from damage to the lymphoid tissue during the collagenase digestion followed by destruction during culture of remaining passenger leucocytes (Simeonovic and Lafferty, 1982b).

These findings that organ culture of donor tissue can reduce immunogenicity and allow allograft survival, are consistent with the Lafferty 2 signal model of graft rejection (Section 1.6.2). That is, the prolongation in survival is not due to the loss of tissue antigens during culture, because such tissues can later be rejected with donor cells or uncultured donor grafts (Lafferty et al. 1976a; Talmage et al. 1976; Lacy et al. 1979c; Simeonovic et al. 1980), but is attributed to the loss of stimulator cells which had been carried as passengers within the graft before culture. The theory of allogenic reactivity (Lafferty and Talmage 1976; Lafferty et al. 1983a) postulates that these passenger cells play two roles in rejection: stimulator cells trigger the T cell response to graft antigens and leucocytes in the tissue are also involved in a nonspecific inflammatory reaction when activated by host stimulating cells. This was shown when cultured BALB/c allografts carried by CBA recipients were retransplanted back to normal BALB/c recipients. While an acute inflammatory reaction caused local damage, it did not lead to rejection of the thyroid graft (Lafferty et al. 1976b; Lafferty and Woolnough, 1977). Bach and his colleagues (Sollinger and Bach, 1976; Sollinger et al. 1977; Gose and Bach, 1979) have confirmed and extended these findings with cultured thyroid xenografts and have also concluded that the immunogenic stimulus is provided for by antigen on metabolically active leucocytes. More recently, Faustman et al. (1981) have demonstrated that treatment of uncultured, isolated adult mouse islets with donor

specific Ia antiserum and complement before transplantation, can result in indefinite graft survival. Because mouse beta cells lack Ia antigens (Faustman et al. 1980; Parr et al. 1980b) the result is further confirmation for the passenger cell concept since it suggests that the antibody and complement treatment of islets removes an Ia or class II bearing passenger cell which is required for cytotoxic T cell activation.

While the organ culture techniques cannot be applied to larger organs such as the kidney, in principle it should be feasible to develop other techniques aimed at removing immunogenic components of such organs. For example, the method of pretreatment of the islets with antiserum and complement (Faustman et al. 1981), is potentially applicable to various organs such as heart, liver and kidney whose parenchymal cells, at least in the rat, have been shown to lack Ia antigens (Hart and Fabre, 1981b). Faustman et al. (1982c) recently extended their earlier findings with islets, by demonstrating a prolongation of mouse heart allograft survival after treatment with anti-donor antiserum and complement.

PART II: TRANSPLANTATION TOLERANCE

1.8 INTRODUCTION

The earliest workers in immunology realised that, as a rule, an individual does not react immunologically to self antigens - a fact formulated in Ehrlich's principle of horror autotoxicus, or fear of poisoning oneself (cited in Boyd, 1966). It is now known that this immunological unresponsiveness or tolerance is not necessarily restricted to self but, under appropriate conditions,

can be extended to foreign antigens.

It should be noted from the outset that there has been considerable semantic confusion in the past concerning the definition of tolerance. The term has been used both in a very broad sense to indicate any condition where an immune response is not manifested, to more restricted meanings depending on particular experimental situations. "Tolerance" was initially used to describe a specific state of unresponsiveness to antigens in adult life as a consequence of exposure to those antigens in utero or during the neonatal period - the latter situation also being termed "neonatal tolerance". Tolerance has also been used interchangeably with such terms as "unresponsiveness", "nonresponsiveness" and "immunologic paralysis". For the purpose of this discussion, an operational definition of tolerance is adopted (Scott and Amos, 1978) which defines tolerance as a specific unresponsiveness or hyporesponsiveness induced by prior exposure to antigen. A total absence of specific reactivity is not implied, but rather, it means a functional failure to achieve rejection.

Because tolerance is a complex immunological phenomenon of great significance, anything more than a bird's-eye view of the subject is beyond the scope of this introduction. Moreover discussion will be restricted mainly to transplantation tolerance. For comprehensive recent reviews on immunological tolerance, see Hraba (1977), Elkins (1979), Hasek and Chutna (1979), and Nossal (1983).

1.9 HISTORICAL

Although the concept of tolerance did not emerge until the classical studies by Medawar and coworkers in the 1950's the term has since been used to describe earlier discovered states of specific inhibition of the immune response. These were seen to have much in common with acquired transplantation tolerance (Billingham et al. 1956a) and include the Sulzberger-Chase phenomenon, immunologic paralysis and tolerance to various heterologous proteins.

In 1929 Sulzberger observed that an intradermal injection (150μg) of neoarsphenamine, which would sensitise guinea pigs, would not do so in animals previously injected intravenously with a larger dose (6mg) of the same antigen (cited in Chase, 1959). Independently, Chase (1946) demonstrated that guinea pigs could be rendered tolerant to the sensitising action of a variety of chemicals, including picryl chloride and dinitrochlorobenzene, by first feeding the substances before the sensitising treatment. This nonreactivity, which came to be known as the Sulzberger-Chase phenomenon (Hraba, 1977) was long-lasting and specific and later also shown to be reversable by adoptive transfers of cells from immune animals (Chase, 1959).

Felton (1949) discovered that while an injection of a relatively small amount (0.5μg) of pneumococcal polysaccharide would immunize mice, the injection of larger doses (for example 0.5mg) did not immunize but rather suppresssed or "paralysed" the ability to form antibody, hence the term "immunological paralysis". Mice so treated were vulnerable to the living organism. The

specificity of this inhibition was later shown in mice where paralysis to one type of pneumococcal polysaccharide did not reduce their antibody forming ability to a polysaccharide of another type (Felton et al. 1955).

At about the same time as the classic studies on the induction of transplantation tolerance (Section 1.10), various workers demonstrated tolerance to heterologous serum proteins (Hanan and Oyama, 1954; reviewed by Chase, 1959). Rabbits given injections of bovine serum albumin (BSA) just after birth had their antibody forming ability suppressed when challenged with the same protein after reaching immunologic maturity (Hanan and Oyama, 1954). Dixon and Maurer (1955) demonstrated that adult rabbits could be made, to use their term, "immunologic unresponsive" if they injected very high doses of the proteins. This effect was specific as it did not prevent antibody responses to closely related antigens. Cinader and Dubert (1955) similarly demonstrated this inhibition of response to human albumin and which they termed "acquired immunologic tolerance". Specificity was again demonstrated by showing that rabbits made unresponsive to human serum albumin, responded normally to tobacco mosaic virus. It should be noted that besides heterologous serum proteins, other antigens such as sheep erythrocytes, bacteria, bacterial lipopolysaccharides, viruses and nucleic acids, have all been used for the induction of tolerance (see Weigle, 1973; Hraba, 1977).

The concept of transplantation tolerance owes its origins to the observations made with dizygotic bovine twins by Owen (1945). He showed that such non-identical

twins have a double population of blood cells and that this "mosaic" or "chimera" was a result of an exchange made between placental blood vessels following anastomoses during foetal life. Moreover, it persists in each cattle twin into adult life. Interestingly, erythrocyte chimerism is not unique to cattle twins for it has also been reported in other animals including sheep (Stormont et al. 1953), chickens from double yolked eggs (Billingham et al. 1956a), marmoset monkeys (Gengozian et al. 1969) and also in man (Dunsford et al. 1953).

The finding of erythrocyte chimerism in cattle provided an important stimulus to Burnet and Fenner (1949) who speculated that tolerance might be induced experimentally. An adult animal does not normally react immunologically to its own tissues because, they suggested, its body cells carry some sort of "self-marker" and the animal learns to recognize self from non-self in embryonic life. Thus, if a foreign antigen can be introduced early enough, the prediction was that the embryo will accept it as part of self and therefore not cause it to develop the capacity of responding immunologically to that particular antigen. Burnet failed, however, to prove his hypothesis experimentally. Using influenza virus, bacteriophage and human blood cells as foreign antigens in the chick embryo, they were unable to detect any change in the subsequent response of the hatched chicks to the antigens (Burnet et al. 1950). Triplett (1962) was later able to show that self tolerance was acquired during ontogeny. He removed the buccal component of the pituitary gland from embryonic tree frogs, Hyla regilla, and then grew the embryo and the gland apart

for about 60 days before regrafting them back together. The now adult gland was rejected because it was recognized not as part of self, but as foreign.

1.10 ACTIVELY ACQUIRED TOLERANCE

Burnet and Fenner's speculation that tolerance might be induced experimentally was given some credence when Anderson et al. (1951) reported that the majority of non-identical cattle twins did not reject reciprocally exchanged skin grafts; control grafts from unrelated calves were rejected normally. These findings led Billingham and coworkers to undertake the experimental induction of transplantation tolerance and were soon able to fulfill the prediction that this tolerance can be established artificially (Billingham et al. 1953). Tolerance was induced in mice by the injection (0.01ml) of an allogeneic viable cell suspension into the embryo. When the embryos were born or later in adult life, they accepted skin grafts from the otherwise incompatible strain. This phenomenon they termed "actively acquired tolerance". Moreover, they demonstrated, firstly, the specificity of this tolerance since CBA mice tolerant of A-strain skin rejected allografts from the unrelated AU strain and, secondly, that it was not an "all-or-nothing phenomenon, but it covered all stages from complete tolerance to normal reactivity. They suggested, however, that animals displaying normal reactivity may have been inadvertantly missed when their litter mates were injected in utero. They also induced similar tolerance in chicks. Cannon and Longmire (1952) had earlier shown that tolerance could be obtained in day old

chicks by direct skin grafting, although the proportion becoming tolerant using this method was only 5-10%. Follow-up studies by Billingham's group in mice, chickens, rats and rabbits confirmed and extended their earlier findings and also demonstrated that immunological reactivity can be easily restored in tolerant animals (Billingham et al. 1956a; Section 1.11).

At the same time that Billingham's group demonstrated acquired tolerance, Hasek independently reported the induction of tolerance to alloantigens in chicks and ducks (cited in Hasek, 1959). He reproduced Owen's phenomena in chickens by using the technique of embryonic parabiosis - the grafting of a whole animal onto another - which gave a prolonged mutual exchange of embryonic blood cells. After hatching, the chickens were separated and able to tolerate mutually exchanged skin allografts. Parabiosis of different species such as duck-chicken and turkey-chicken, produced only a moderate prolongation of skin graft survival (Hasek, 1959). Billingham et al. (1956a) using similar techniques, found that a similar low degree of tolerance existed in duck-chicken parabionts. That is, transplantation tolerance is much more difficult to induce with xenogeneic differences. For an early review of tolerance induction using parabiosis, see Simonsen (1962).

Woodruff and Simpson (1955) using rats were able to confirm the early findings of Billingham et al. (1953). Moreover, they demonstrated that, in the rat, unlike the mouse, the period during which tolerance may be conferred extends beyond the foetal stage for up to 2 weeks after birth. In the mouse this period corresponds roughly with

foetal life.

The types of cells injected into the foetus or neonate were also shown to be important. When tolerance was induced with non-immunologically reactive cells such as foetal or adult testicular or kidney cells, the resultant tolerant animal showed no pathological changes. When injections of adult spleen cells and blood cells were used, the immunologically competent cells became sensitised. Because the host cannot respond, a GVH reaction results which can lead to the death of the host. In intermediate cases the reaction, termed "runt disease", causes stunted growth (Billingham and Brent, 1957; Simonsen, 1957; reviewed by Hasek et al. 1961). This ability of injected cells to give a GVH reaction is a major impediment in clinical bone marrow transplantation.

The term "actively acquired tolerance" given by Billingham et al. (1953), is an apt description because it, firstly, defined tolerance as that of the graft by the host and, secondly, stated that, like acquired immunity, this tolerance was also an outcome, albeit an opposite one, of the encounter with antigen. Their explanation for it was that cells which are able to produce an immune reaction to the tolerated graft are absent in the tolerant animal. Accordingly, tolerance was defined as "the specific and systemic failure of the mechanisms of immunological response which is brought about by exposing embryos or very young animals to 'antigenic' stimuli" (Billingham et al. 1956a).

This work also influenced Burnet (1959) who explained tolerance on the basis of his clonal selection hypothesis

of antibody formation. Briefly, his hypothesis stated that an organism has a large pool of potentially reactive lymphoid cells, each of which is capable of dividing after contact with a specific antigen and which would then proliferate into clones making only one kind of antibody. During embryonic development, the encounter of young lymphoid cells with self antigens or indeed with injected antigens, results in the elimination of those cells. Therefore, tolerance results from an elimination of specific alloantigen reactive clones in the recipient during a critical state of immunological development. Put another way, transplantation tolerance occurs, to use Burnet's words, when "the content of self-components in the body has been enlarged by an experimental manipulation" (Burnet, 1961).

The conventional view of transplantation tolerance therefore, has been one based on the clonal elimination of the reacting cells. However, work in the early 1970's on blocking factors and suppressor T cells were an obvious challenge to this view (Section 1.14).

1.11 DURATION AND ABOLITION OF TOLERANCE

The induction, degree and duration of transplantation tolerance depends on a number of factors which include the species and strain of the animals used, source of cells or antigen and its dose, route of inoculation and the immunocompetence of the host (reviewed by Weigle, 1973; Elkins, 1979). Some of these points have already been covered in Section 1.10. Suffice to say that once tolerance is induced, there is a requirement for the persistence of antigen (the tolerogen), for the mainten-

ance of tolerance, otherwise it will terminate spontaneously (Weigle, 1973). For example, if tolerance of non-replicating antigens such as serum proteins is to be maintained, repeated administration of the antigen is necessary (Dixon and Maurer, 1955).

Medawar and Woodruff (1958) reported a weakening of tolerance of skin grafts following removal of the established grafts and noted that a second allograft (transplanted one month later) from the same donor was rejected. Where tolerance is not complete, allograft survival may be considerably prolonged but eventually it will be rejected (Hasek, 1959). Irrespective of the degree of tolerance induced, however, tolerance can be readily abolished.

The abolition of tolerance to heterologous serum proteins has been demonstrated by the injection of antigens closely related to the tolerogen. Thus, tolerance to human serum albumin was terminated by injecting an azobenzene derivative of albumin (Cinader and Dubert, 1955), and to BSA by administering serum albumin from various related species (Weigle, 1961). Curtain (1959) was able to terminate tolerance to Bence-Jones protein by injecting myeloma proteins from the same individual.

The abolition of transplantation tolerance has been achieved using several methods including irradiation (Fefer and Nossal, 1962), but more commonly through the adoptive transfer of normal or sensitised lymphocytes. Billingham et al. (1956a) were able to reject CBA skin grafts in tolerant A strain recipients by adoptive

transfer of both immune and normal lymph node cells from other A-strain animals. Tolerance abolition was much more prompt, however, with immune cells. A similar finding was reported by Kilshaw et al. (1974) using tissue extracts and ALS to induce tolerance (Section 1.12). Hasek and Chutna (1979) also confirmed these findings and further found that the dose of immunocompetent cells make no difference to abolition. Billingham's group interpreted this abolition as an indication that tolerance represents a "central failure" of the immune response and cannot be explained by a peripheral interference of the allograft reaction by processes such as antibody absorption or by graft adaptation (Billingham et al. 1956a).

1.12 INDUCTION OF TOLERANCE IN ADULT ANIMALS

Although transplantation tolerance has been successfully induced in neonates in various experimental systems there has been little success in finding a truly clinically applicable transplantation technique in adult animals. Prolonged allograft survival and induction of tolerance in adult animals has usually required modification of the host's immunocompetence. This has been achieved using such methods as immunosuppressive drugs, antilymphocyte serum, irradiation, thoracic duct treatment, thymectomy and combinations of any of these. In addition, pretreatment of recipients with donor tissue extracts and blood products have also been used. In our laboratory we have used a combination of cultured islet allograft and treatment of the recipient with ultraviolet irradiated spleen cells of donor origin to induce adult tolerance (Chapter 6). Many experimental procedures which give a measure of

specific tolerance, mainly in rats and mice, have been described and early studies have been reviewed by Weigle (1973). Many of these techniques have also been used clinically but despite varying success, the resultant infectious, neoplastic, pharmacologic or radiologic side effects have yet to be adequately controlled. Various grafts have also been protected by transfer of hyper-immune serum and this will be discussed in Section 1.13.

1.12.1. Immunosuppressive drugs

This is a rather broad topic and beyond the scope of this review. However, because of the clinical importance of the various cytotoxic drugs and steroids used for prolonging allograft survival, a brief summary is given. It should be stressed that without such drugs, much of the current clinical transplantation would not be possible. For a recent review of immunosuppressive drugs, see Salaman, 1983.

Graft rejection was and remains the primary obstacle. One widely used drug has been azathioprine. For example, Ballinger and Lacy (1972) reported the first successful transplantation of isolated islets in rats using this drug. The use of corticosteroids, azathioprine and other such drugs, however, is limited by their side effects and by the danger of reducing the patient's ability to combat infections (Opelz and Lenhard, 1983). By combining drugs, an accummulation of toxic effects may result. Floersheim (1969) combined several drugs, including cyclophosphamide, methotrexate and azathioprine to prolong BALB/c skin survival in CBA mice. Drugs have also been combined with other treatments such as ALS (Section 1.12.2) and thymectomy (Chernyakhovskaya et al. 1980).

In recent years cyclosporine (CyA) (also referred to as Cyclosporin A) has been shown to be a very promising and important immunosuppressant in numerous experimental studies and clinical trials carried out since its extraction and purification in the mid 1970's. Unlike other drugs, CyA is primarily lymphocyte specific and also has a very low degree of myelotoxicity (Thomson, 1983). These features make its clinical use most attractive, particularly in bone marrow transplantation. Because it does not affect the migratory capacity of phagocytic cells, its use ensures that the anti-microbial defences of the recipient are not completely destroyed. The immunobiology, mechanism of action and clinical use of this drug, have recently been extensively reviewed (Britton and Palacios, 1982; White and Calne, 1982; Lafferty et al. 1983b; Thomson, 1983). In addition to its immunosuppressive use, CyA is proving a valuable analytical tool in the study of the role of T cell subpopulations in cell mediated immunity (Borel and Lafferty, 1983; Chapter 5).

1.12.2. Antilymphocyte serum

Antilymphocyte serum (ALS) - an immune serum raised in one species against the lymphocytes of another species - has been used both experimentally and clinically for many years. Numerous reports have shown ALS to prolong survival of skin, renal, hepatic and cardiac allografts in experimental animals across major histocompatibilities (reviewed by Lance et al. 1973; Wonigeit and Pichlmayr, 1977).

The effect of ALS on allograft survival can be enhanced by combining ALS with procedures such as thoracic duct drainage (Woodruff and Anderson, 1963) and thymectomy (Steinmuller, 1979). Others have combined bone marrow cells and ALS to induce tolerance of skin allografts in mice (Monaco et al. 1971; Wood et al. 1971, 1972). Synergy has sometimes been achieved by combining ALS with various cytotoxic drugs. Floersheim (1969, 1973), and Brent and Opara (1979) reported that ALS treatment combined with procarbazine gave prolonged skin and heart allograft survival in mice. This synergy was also demonstrated with ALS treatment combined with splenic and liver cell extracts (Brent and Kilshaw, 1970; Brent et al. 1971). Pinto et al. (1974) achieved long term survival of skin allografts by the administration of a combination of liver or spleen extracts, ALS and Bordetella pertussis vaccine. The effect was strain specific. Moreover, these workers found that induction of tolerance was dose dependent on both the extract and ALS, as well as on the timing of ALS injections (Brent et al. 1971, 1973). Marquet and Heystek (1975) were able to gain considerable pancreatic islet allograft survival in rats by using a short ALS treatment.

Although ALS has been shown to prolong allograft survival in several animal models, there are conflicting data as to whether it is able to prolong the survival of renal, hepatic or cardiac allografts in human recipients (reviewed by Heyworth, 1982). This conflict may, in part, be explained by the different types and doses of ALS which have been used clinically by different

workers. In addition, assessment is also complicated because of other immunosuppressive agents used and by the different patterns of treatment given to suit the needs of different patients. Heyworth (1982) is of the opinion that for human renal allografts, the clinical experience with ALS has not convincingly demonstrated prolonged survival. In any case, the evidence that ALS does have marked immunosuppressive activity in humans, is not without its complications. The question of side effects of ALS in clinical use has been reviewed by Lance et al. (1973).

The development of suitable monoclonal antibodies which, unlike conventional antisera, are well defined, may become a better method of immunosuppression (Williams, 1979; Diamond et al. 1981; Millstein , 1981). Cosimi et al. (1981a) recently undertook a trial of monoclonal antibody therapy in human renal allograft patients. Eight such patients had their rejection crisis reversed with OKT 3 monoclonal antibody (reactive with all mature T cells). These workers have also used this approach to monitor the patients' immunologic status (Cosimi et al. 1981b). By using flow cytometry and monoclonal antibodies to T cell subsets, they were able to monitor changes in patients' peripheral blood lymphocytes including total numbers of T cells (using OKT3), helper T cells (OKT4) and cytotoxic T lymphocytes (OKT8).

While there is considerable evidence that ALS depletes E-rosette-positive lymphocytes in humans (Heyworth, 1982), the precise mechanism remains unknown. Kilshaw et al. (1975) have suggested an important role

for suppressor T cells in maintenance of tolerance following ALS and tissue extract treatment. They found that adoptive transfer of 2×10^8 spleen cells from tolerant CBA mice to normal syngeneic mice and subsequent grafting with A strain skin, produced a significant prolongation in skin survival. CBA female recipients injected with tolerant viable spleen cells (1.4×10^8) showed an impaired ability to reject A-strain tumours and treatment with anti-θ antibody and complement abolished suppressor activity from tolerant spleen cells (Kilshaw et al. 1975). Other mechanisms may be involved and these are discussed in Section 1.14.

1.12.3. Irradiation

Whole body irradiation (WBI) has been used for inducing tolerance to bone marrow and kidney allografts although high doses of irradiation produced unacceptable side effects such as irreversible bone marrow aplasia (Slavin et al. 1980). The impact of ionizing irradiation on the immune system in clinical transplantation has been of some concern because of the many detrimental effects on immunological defence mechanisms which increases the risks of infectious diseases (reviewed by Serrou and Rey, 1981; Doria et al. 1982). As a result, more selective approaches to the use of irradiation have been stimulated. Strober and coworkers have recently revived interest in the use of irradiation by the introduction of total lymphoid irradiation (TLI; Strober et al. 1979). This technique is based on that used to treat human lymphoid malignancies such as Hodgkin's disease (Kaplan, 1981) and uses X-irradiation of the

lymphoid organs only. Animals are given daily doses or fractions (200 rads) while shielding bones, lungs and most of the gastrointestinal tract, until a total dose (usually 3400 rads) is reached before the animal is grafted. Using this technique, the induction of specific transplantation tolerance has been reported for skin and heart allografts in mice and rats (Slavin and Fuks, 1979; Strober et al. 1979). More recently TLI has been used to establish specific and long-lasting tolerance of bone marrow without GVH (Slavin et al. 1980). Vaiman et al. (1981) used an adaptation of this protocol to obtain prolongation of skin allograft survival in pigs. Strober's group also combined TLI with CyA or anti-thymocyte globulin in the hope of demonstrating a synergistic effect on cardiac allograft survival in monkeys but failed (Pennock et al. 1981). However, CyA alone or in combination with TLI was associated with the development of malignancies. Using a reduced total irradiation, the TLI method was equally effective in inducing tolerance to liver and kidney allografts in baboons (Myburgh et al. 1980a,b).

The mechanism of action is uncertain but evidence suggests the presence of suppressor cells. Spleen cells from TLI chimeras were assayed for their ability to suppress the MLR of responder lymph node cells and were found to suppress the response for prolonged periods after bone marrow transplantation (Strober et al. 1979). Myburgh et al. (1980a,b) also demonstrated in vitro evidence of suppressor cell activity although much of it was nonspecific. The presence of nonspecific and donor specific suppressor cells following TLI was also

shown by the inhibition of MLR after the addition of mitomycin treated peripheral blood lymphocytes from treated animals (Smit et al. 1980, 1981; Myburgh et al. 1983).

Another selective irradiation approach has been used by Hardy et al. (1979) using the systemic administration of Palladium (Pd)-109-hematoporphyrin. However, no significant effect was demonstrated on rat heart allograft survival, although its combination with a short course of ALS gave prolonged survival.

1.12.4. Blood pretreatment

Although the effect of blood pretreatment and transfusion on transplantation is highly variable, it has been widely used both clinically and experimentally in the hope of inducing tolerance, or at least prolonging survival in adult recipients. Early studies include those of Halasz et al. (1964) who demonstrated some prolongation of kidney allograft survival in mongrel dogs following pretreatment of recipients 10 and 5 days before transplantation with 2ml of blood from the prospective kidney donor, and Marquet et al. (1971) who achieved marked prolongation of survival for kidney and heart allografts in rats which had been pretreated with small quantities of donor blood. A similar effect was demonstrated by Fabre and Morris (1972) with rat kidneys. Attempts by them to transfer tolerance by the passive administration of serum were unsuccessful. More recently, Faustman et al. (1982a,b) obtained long term survival of islet allografts in mice by treating recipients with donor blood treated with antiserum to class II antigens, and Lau et al. (1983) demonstrated prolonged islet survival in rats by treat-

ment with blood irradiated with ultraviolet (UV) light.

Clinical investigations were at first discouraged because it was early recognized that antibodies, as a result of blood transfusions, can cause hyperacute rejection (Kissmeyer-Nielsen, 1966; Section 1.5.1.1). However, Opelz et al. (1973) found that contrary to the then common view, pretransplant blood transfusions improved renal allograft survival. Many independent studies have since been undertaken and various prospective trials have confirmed those of retrospective studies indicating that transfusions do improve the transplantation survival rate (Fehrman et al. 1980; Opelz et al. 1981a; reviewed by Opelz and Lenhard, 1983).

These clinical findings were also subsequently confirmed in a number of animal studies which combined blood pretreatment with various levels of immunosuppressive treatment. These include skin allografts in mice (Okazaki et al. 1980; Horsburgh et al. 1981; Wood et al. 1981), islet allografts in rats (Selawry et al. 1981), and kidney allografts in dogs (Obertop et al. 1978) and monkeys (Van Es et al. 1978).

Despite the risks of hepatitis transmission and the sensitisation of potential recipients, particularly when many transfusions have been administered, most clinical workers would now agree that pretransplant transfusions will improve renal allograft survival. The benefits are seen to outweigh by far the risks involved and transfusions with random blood have become mandatory in most clinical transplant centers (Opelz and Lenhard, 1983; Van Rood, 1983).

The use of donor-specific blood in recipients of

living related kidneys may, in the future, assume greater importance. Cochrum et al. (1981) used transfusions from the actual kidney donor in HLA-mismatched related-donor transplants and were able to obtain graft survival rates comparable to those pretreated with random blood transfusions. In experimental studies with mice, conflicting results have been obtained. While Okazaki et al. (1980) demonstrated that prolongation of skin graft survival was induced only by nonspecific blood transfusions and not by specific blood transfusion, Wood et al. (1981), in contrast, found that small quantities (0.5-2µl) of donor specific blood can induce long lasting survival of skin allografts. This difference may reflect a difference in blood dose since, when higher volumes (0.1ml) of blood were used, donor specific blood did not induce tolerance (Wood et al. 1981).

Despite the wide use of random blood transfusions for clinical kidney transplantation, there is still a lack of consensus on transfusion protocols, and consequently a number of controversies and discrepancies exist among the published results. Considerable variations exist, for example, in number and interval of transfusion. While some workers have found that patients who received many transfusions did better than those who received only a few (Fehrman et al. 1980, 1982; Opelz and Terasaki, 1980), others have reported that even a single dose or very few transfusions were adequate to achieve the maximum benefit (Williams et al. 1979; Feduska et al. 1981; Persijn et al. 1981). The interval between transfusions has varied ranging from several months to just a few hours, but this variation apparently does not matter

(Opelz et al. 1981b; Fassbinder et al. 1982). Van Es et al. (1978) noted that a single blood transfusion given 0-12 hours before kidney transplantation, prolonged allograft survival in monkeys. Even transfusions given during transplantation surgery have been claimed to be effective by some investigators (Williams et al. 1980), although in series with large numbers of patients and regardless of the number of such transfusions, this could not be confirmed (Opelz and Terasaki, 1980, 1981). While most would agree that blood depleted of white cells is not effective (Wood et al. 1981; Opelz and Lenhard, 1983), in rhesus monkeys it was recently reported that donor platelets were as effective as whole blood transfusions (Borleffs et al. 1982).

The mechanism involved in the beneficial effect of blood transfusion remains obscure. Many explanations have been proposed (reviewed by Opelz and Van Rood, 1983) and it now seems that several mechanisms may be involved. Some have agreed that this effect in human renal transplants can be explained simply by a selection mechanism, that is, patients with a positive lymphocytotoxic crossmatch test are automatically excluded (Opelz et al. 1972). However, the extensive data analysed by Opelz and coworkers suggests that although this form of selection may play a role, it cannot account for the entire beneficial effect (Opelz et al. 1981a; Opelz and Terasaki, 1981). Several immunological mechanisms have been proposed, the most important of which appear to be the induction of suppressor cells (Maki et al. 1981; Marquet and Heystek, 1981;

Lenhard et al. 1982) and antiidiotypic antibodies (Miyajima et al. 1980; Singal et al. 1982), with the former being favoured by the majority of investigators (Opelz and Van Rood, 1983; Section 1.14).

1.13 IMMUNOLOGICAL ENHANCEMENT

Immunological enhancement is the prolonged survival of an allograft as a result of the presence in the recipient of either endogenous or passively transferred antidonor alloantibody. The terms "active enhancement" and "antigen-induced suppression" have been used synonymously for the former situation where antibodies have been produced in the host in response to the injection of preparations of donor tissue, and "passive enhancement" or "antibody-induced suppression" for the latter.

The concept of enhancement arose from the work on tumour allografts by Casey and his colleagues in the 1930's (cited in Woodruff, 1960). They demonstrated the prolonged survival of tumour allografts in recipients which were pretreated with injections of similar tissues and which had first been killed by lyophilization and other methods. This phenomenon was termed the "enhancing effect," the "XYZ effect" and the "conditioning the host effect". Kaliss and Molomut (1952) found they could obtain the enhancement of tumour allografts by the passive transfer of serum and later work demonstrated this phenomenon was dependent on the presence of antibodies in the serum (Kaliss and Bryant, 1958). Mitchison and Dube (1955) reported that like actively acquired tolerance, enhancement also could be abolished with immune cells from the regional lymph nodes but, unlike

tolerance, could not be abolished with normal cells. Billingham et al. 1956b) performed studies with mouse skin allografts and showed that enhancement was not restricted to neoplastic tissues.

Since the mid 1950's, numerous investigations have reported the survival of both tumour and normal tissue allografts as a result of either active, passive enhancement or a combination of both. While various model systems have been used with resultant variations in the effectiveness of the enhanced state, the immunological status of recipients of long-term surviving allografts is very similar and unlike classical tolerance, in general lymphoid cells from long-term recipients retain the ability to react normally in vitro to donor-type antigens (Feldman, 1972; Carpenter et al. 1976; Stuart et al. 1979, 1980).

The relationship between enhancement and tolerance has been a source of some controversy. While Snell (1954) proposed that the term "actively acquired tolerance" should be extended to include enhancement, Billingham et al. (1956b) argued for a distinction between the two phenomena on the basis that the means of achieving them and probably the mechanisms involved, were different. The unresponsiveness in classical tolerance was seen to be due to an antigen induced state which led to the elimination or inactivation of the clone of cells capable of responding to the specific antigen (Section 1.10). In contrast, enhancement was seen as an antibody-induced state resulting in the protection of the antigen-bearing target cells. However, the distinc-

tion between classical tolerance and immunological enhancement was becoming less clear by the 1970's with the work, for example, of the Hellstroms (Hellstrom and Hellstrom, 1974) who proposed an alternative mechanism to clonal deletion, namely, that tolerance depends on circulating serum-blocking factors (Section 1.14). This suggested that tolerance and enhancement were part of a single spectrum. Recent developments which have made an impact on our understanding of tolerance and enhancement are investigations into the role of class II antigens, suppressor T cells and anti-receptor or anti-idiotypic responses in the induction and maintenance phases of immunological suppression (Section 1.13.6).

This discussion will be restricted to enhancement of normal tissue allografts. For recent reviews see Wonigeit and Pichlmayr (1977), Stuart et al. (1979, 1980) and Morris (1980).

1.13.1. Active enhancement

Active enhancement is less predictable than passive enhancement and is more likely to result in accelerated rejection rather than enhancement (Stuart et al. 1979, 1980). Experimentally, antigen treatment with and without the addition of non-specific immunosuppressive agents such as ALS and various drugs, has been used to induce prolonged survival in various models. These include skin allografts in mice (Brent and Kilshaw, 1970; Brent et al. 1971; Section 1.12.2) and renal allografts in the rat (Ockner et al. 1970) and dog (Zimmerman et al. 1968), although Calne et al. (1966) were not able to prolong kidney allograft survival in dogs after treat-

with a variety of different antigen preparations, with or without immunosuppressive drugs. Mullen (1980) could gain a marked prolongation of foetal pancreas allograft survival in rats only by combining donor antigen with ALS and procarbazine hydrochloride.

1.13.2. Passive enhancement

Because enhancement does not involve a generalised depression of immune responses, its potential for clinical application was quickly realised. But although tumours were readily enhanced by the passive administration of antibody (Kaliss amd Molomut, 1952), similar attempts using skin allografts were generally unsuccessful and, consequently, clinical interest in the phenomenon waned. With the development of microvascular surgical techniques in the 1960's, which enabled the grafting of vascularized grafts such as heart and kidney, passive enhancement was again reexamined. The rat renal allograft model became widely used. Stuart et al. (1968) were the first to demonstrate prolonged survival of kidney allografts in rats following treatment with both donor antigen and passive transfer of anti-donor alloantibody. French and Batchelor (1969) confirmed this enhancement in AS rats using only a passive transfer of antiserum. Numerous other studies soon followed which confirmed and extended these findings in the rat renal allograft model (French et al. 1971; French and Batchelor, 1972; Fine et al. 1973; Mullen et al. 1973; Fabre and Morris, 1974, 1975; Fabre and Batchelor, 1975; Winearls et al. 1980).

Donor specific alloantiserum is usually administered intravenously either at or shortly after transplantation,

and quite small doses - as little as 50µl for 160-200gm rat (Fabre and Morris, 1973) - are sufficient to enhance rat kidney allografts. The enhancing antisera have been raised in various ways, but generally it is done by hyperimmunization of the recipient with donor lymphoid cells (Stuart et al. 1979).

While the passive enhancement of renal allografts has been shown to be achieved consistently in the rat, in other species it has been both more difficult to achieve and runs the risk of inducing hyperacute rejection (McDowall et al. 1973).

Variation in the effect of enhancement also exists with strain combination and type of allograft. For example, in the Lewis to DA rat strain combination, passive enhancement prolongs renal allograft survival to >300 days, whereas in the August to AS, the mean survival is 43 days; untreated recipients undergo acute rejection, surviving only 12-14 days (Morris, 1980). The type of tissue used for transplantation also has a strong influence on the effectiveness of enhancement, with variations existing for different tissue allografts in the same strain combination. Generally, kidney allografts may survive indefinitely while heart allograft survival is moderate and skin is marginal (Tilney and Bell, 1974; Nash et al. 1977). Islet allografts behave somewhat similarly to skin. Finch and Morris (1976) found that while about half of the pancreatic islet allografts in rats could be enhanced using the F_1 hybrid donor, rejection could not be delayed with homozygous islets. Nash et al. (1977) compared the enhancement of rat islets with heart, kidney and skin allografts. Prolongation in survival was best with kidney and then heart,

islet and skin in descending order. Only 4/10 islet allografts showed some prolongation in survival. More recently Reckard et al. (1981) confirmed this spectrum of increasing immunogenicity in rats, with renal allografts being least immunogenic, followed by heart, segmental pancreas and isolated islets.

The reason for this degree of variation with different tissues is not known. Differences in antigenic composiiton have been suggested (Lance et al. 1973). Skin specific antigens (Sk antigens) were thought to be responsible for the difficulty experienced with skin allografts (Steinmuller and Lofgreen, 1977) but even if enhancing sera are raised with skin grafts instead of the usual hyperimmunization with lymphoid cells, there is still relatively little improvement on the survival (Morris, 1980). The great variation in amount of class II antigens expressed on different tissues, might account for the variation in enhancement of different tissues (Hart and Fabre, 1979; Davies and McKenzie, 1980).

Clearly, then, the rat is not only the most thoroughly investigated model but it is also the experimental animal in which enhancement of vascularized organs seems to be most easily achieved, whether using active or passive enhancement or a combination of both. The few clinical studies attempted have not given convincing evidence of improvement in kidney graft survival in man and, according to Fabre, much of the clinical hope of enhancement has now faded (Fabre, 1982).

1.13.3. Class and subclass of enhancing antibody

The evidence indicates that the class of antibody conferring enhancement is confined to the IgG class (Mullen and Hildermann, 1971; Carpenter et al. 1976; Mullen et al. 1977) although Voisin et al. (1969) have reported enhancing activity by the IgA class in mice. IgM does not enhance and has in fact been shown to be cytotoxic, thereby reducing the length of survival of rat kidney allografts (Mullen and Hildemann, 1971; Mullen et al. 1977).

Jansen et al. (1975b) obtained pure preparations of mouse 7S IgG subclass by means of affinity chromatography and demonstrated that IgG_1 and IgG_2 were both able to enhance skin allografts in mice, although only the latter subclass was also able to induce both enhancement and hyperacute rejection. In rat kidney allografts, only IgG_2 has been shown to induce enhancement (Mullen et al. 1977). Duc et al. (1982) found that the Sa 1 mouse tumour can be enhanced with IgG_1 but not IgG_2.

1.13.4. Specificity of enhancing antibody

Early studies with tumour (Moller, 1963) and skin (Jeekel et al. 1972) allografts in the mouse suggested a strong specificity of the enhancing sera. Later studies using the rat kidney model, however, indicated considerable cross reactivity or lack of specificity (Fabre and Morris, 1974; Fabre and Batchelor, 1975), although more detailed serological studies by Morris' group have since shown that the cross reactivity is less than what had been previously thought (Morris, 1980).

1.13.5. Target antigen of enhancing antibody

One of the most important questions to arise from studies of enhancement of normal tissue allografts has been whether the enhancing activity is associated with antibody directed towards products of one or several regions of the MHC. Following the demonstration by Davies and Alkins (1974) that enhancement of rat heart allografts could be achieved by antisera absorbed with erythrocytes, which express class I antigens only and not by antisera absorbed with spleen and thymus cells, which are both class I and II positive, they developed the view that passive enhancement was produced by the anti-class II antibody in the enhancing antisera. The removal by absorption of antibodies directed at class II antigens is not formal proof, however, that class I antibodies are not able, on their own, to enhance graft survival. Nonetheless, a number of studies using both absorption techniques and recombinant strains, soon followed which supported these findings. Class II antibodies were confirmed in mouse skin (Staines et al. 1975; McKenzie and Henning, 1977) and kidney allografts (Soullilou et al. 1976). In an extensive review of rat and mouse experimental studies, Davies and Staines (1976) concluded that anti-class II antibodies were sufficient to achieve passive enhancement of rat heart and mouse skin allografts, and Winearls et al. (1980) have more recently demonstrated this with rat renal allografts.

Others, however, have since reported that anti-class I activity is sufficient to enhance. Gallico et al. (1979) demonstrated with a rat recombinant haplotype that kidney allografts can be enhanced by antibodies directed at the products of

either class I or class II regions. More recently, Davies and coworkers have themselves shown that heart allografts in mice can be enhanced to a similar extent with antibodies to the products of either class I or class II regions, although skin could only be enhanced with anti-class II (Davies and McKenzie, 1980). De Waal et al. (1980) reported that both anti-H-2K and H-2D or class I allo-antibodies have a weak but significant protective effect in mouse skin allograft survival. More recently still, Duc et al. (1982) showed that mouse SA 1 tumour allografts can be enhanced with anti-class I antibody, although anti-class II antibodies have some activity on Sa 1 cells when grafted on C57BL/Ks mice.

Thus, antibodies against donor class II antigens are not uniquely involved in enhancement as earlier believed. It is clear that both class I and class II antigens can induce enhancement depending on the models used and the techniques applied. This may even suggest that there are a number of ways in which the antiserum acts to induce enhancement.

1.13.6. Mechanisms of enhancement

The question of mechanisms by which enhancing antibodies prolong allograft survival has been the subject of considerable controversy and speculation, and the whole issue is complicated by the use of different tissues and organs, different protocols and different rejection criteria. Early views on mechanisms tended to apply a single mechanism and often equated induction with long term enhancement. For example, Billingham et al. (1956b) suggested that antiserum prevents or delays the graft

antigens from reaching the regional lymph nodes, thereby preventing a cellular response. This inhibition was called an "afferent inhibition" and which Snell (1957) called a "walking off" of the graft. This idea is based on the division of the locus of effect of the rejection response at 3 levels: afferent, central and efferent. Billingham argued that enhancement cannot be efferent inhibition because it cannot be induced in animals which are already immunized with living cells. Central inhibition also seemed to be out since Mitchison and Dube (1955) failed to abolish enhancement with normal lymph node cells - a procedure which could abolish actively acquired tolerance which was considered to be due to a central inhibition (Billingham et al. 1956a). In contrast, Kaliss and Bryant (1958) argued for a form of efferent inhibition in which the antibodies somehow lead to an adoptive change in the graft and, as a result, the graft survives. One of their main arguments against the concept of afferent blockage was that immunity can be detected at the same time and in the same recipient carrying an enhanced graft. Graft adaptation has been repeatedly suggested (Woodruff, 1952, 1954, 1960; Cannon, 1957) and is discussed in Section 1.13.6.2.

However, the mechanisms of afferent, central and efferent blockage need not be mutually exclusive, as one or more may be operating at various times during the survival of an enhanced graft. Any discussion of mechanisms of enhancement, therefore, ought to make a distinction between at least two phases: the induction phase in which the immune reaction is either delayed or suppressed, and the maintenance phase which enables long-

term or indefinite graft survival.

1.13.6.1. Induction phase of enhancement

The induction phase might operate peripherally such as by antigen masking or "coating" (Morris and Lucas, 1971), which would delay or prevent antigen recognition, or via a central mechanism which requires the active suppression of the immune response after antigen recognition has occurred (Morris, 1980). The basic assumption behind ideas of peripheral blockade is that such a process leads to a complete masking of the antigenic determinants of the graft by the enhancing antisera and consequently inhibits the interaction of recipient lymphocytes with donor antigens. However, a peripheral effect by antigen masking is unlikely because, firstly, it has been shown that enhancement can be induced with very small (50µl) amounts of antisera (Fabre and Morris, 1973) and, secondly, passively administered alloantibody disappears very rapidly (French and Batchelor, 1972; French, 1973).

The evidence seems to favour a central mechanism and three main ones have been suggested. The first is based on the role of anti-class II antibody which could directly affect the passenger leucocyte component (Davies and Staines, 1976; Soulillou et al. 1976). Passenger leucocytes are class II positive and play a key role in the induction of an immune response (Section 1.6). Another idea is that antigen-antibody complexes interfere with the central part of the rejection reaction (French and Batchelor, 1972; Wonigeit and Pichlmayr, 1977). The precise mechanism of their blocking effect is not known but several possibilities have been suggested

(see Theofilopoulos, 1980), including opsonization and phagocytosis. Hutchinson has proposed that antigen-reactive cells are opsonized by graft antigen-antibody complexes and are subsequently destroyed by Fc receptor-bearing host cells such as macrophages (Hutchinson, 1980; Hutchinson and Brent, 1981). Hutchinson (1980) has reviewed the data in support of this hypothesis and their most direct evidence comes from their studies in which syngeneic radiolabelled antigen reactive cells were taken up by macrophages when injected intravenously into rats carrying enhanced renal allografts, or mice carrying enhanced skin allografts. However, opsonization is obviously not so complete as to remove all antigen reactive cells because normal proliferative and cytolytic responses occur in in vitro assays using lymphocytes from enhanced graft recipients (Stuart et al. 1980). The proportion and subpopulation of host T cells which bind to the antigen need to be demonstrated before adequate assessment of the relative importance of this mechanism can be made. A third possibility might be that the passively administered alloantibody induces antibody formation to the antigen combining site of the antibody itself, that is, anti-idiotypic antibody. This antibody would also react with receptor sites for that antigen on T lymphocytes and thus again block the induction of the immune response at a central level. For example, Stuart et al. (1976) induced Lewis rats to make antibody against Lew anti-Brown-Norway (L anti-BN) by immunization of Lewis rats with complexes of BN antigen and anti-BN antibody. They demonstrated that the acceptance of LBN kidneys by Lewis recipients occurred

when transplants were performed at 10 days post treatment with antigen and antiserum, that is, when the titre of L anti-(L anti-BN) idiotypic antibody was at its peak. Kidneys transplanted either before the appearance or after the disappearance of anti-idiotypic antibody were rejected.

At present then, the mechanism by which the induction of enhancement might operate remains speculative and it is also possible that more than one mechanism might be operative.

1.13.6.2. Maintenance phase of enhancement

Once enhancement is induced, either passively, actively or by a combination of both, the maintenance phase seems to require an active process on the part of the recipient. This phase is much more complex and may depend on more than one mechanism. Three general mechanisms have been proposed for this phase: clonal deletion, graft adaptation, and regulation in some way of the host's response to the allograft.

Clonal deletion proposes that a specific clone of antigen reactive cells are deleted during the induction of enhancement, thus allowing a stable maintenance phase. However, existing evidence provides little support for this mechanism. For example, the injection of syngeneic lymphocytes into recipients carrying long surviving kidney allografts did not lead to rejection (McKenzie and Morris, unpublished, cited in Morris, 1980). Moreover, spleen cells from similar recipients proliferate normally in MLC (Stuart et al. 1979) and produce a normal GVH reaction in a popliteal lymph node assay (French et al. 1971; Mullen et al. 1973).

Graft adaptation has long been suggested as a

mechanism for allograft protection following the Woodruffs' demonstration that guinea pig thyroid allografts which remained in the anterior chamber of the eye for some time, remained invulnerable when retransplanted to non-privileged sites in the body (Woodruff and Woodruff, 1950). To explain this phenomenon, Woodruff (1952, 1954) formulated the hypothesis of the "critical period" according to which allografts became progressively less vulnerable with time and after a certain critical period are capable of surviving indefinitely in the host. Although this idea was criticised by those who saw tolerance as an adaptation of the host (Billingham et al. 1956a), some support has been given in more recent years (Sharav et al. 1969; McKenzie et al. 1972; Warden et al. 1973; Hart et al. 1980). Batchelor's group was able to demonstrate indefinite survival when long term surviving kidneys in the (AS x Aug) F_1 to AS and (ASxWF) F_1 to Lewis rat strain combinations were retransplanted to fresh AS and (ASxWF) F_1 recipients respectively (Batchelor et al. 1979; Welsh et al. 1979). They attributed the lack of response to the retransplanted kidney to the absence of passenger leucocytes in the enhanced kidney - a conclusion also arrived at in an extensive study by Hart et al. (1980). Antigenic deletion or modification has been suggested to account for adaptation in tumour studies (Woodruff and Symes, 1962; Boyse et al. 1963) but this has not been demonstrated for normal tissues. Antigen alteration in the enhanced kidney is unlikely since there is evidence that long surviving grafts carry a normal complement of MHC antigens as measured by antibody absorption (French, 1972) or ^{125}I labelled antibody

uptake (Fine et al. 1973). Moreover, antigen in sufficient amounts to lead to allograft rejection, persists in the enhanced graft following retransplantation (Stuart et al. 1970), although this persistance varies with the strain combination used (Hart et al. 1980). Using radioimmune binding assays, Hart et al. (1980) have also demonstrated that there is no qualitative difference in the amount of class I or II antigens in long surviving (DA x Lewis) F_1 kidneys compared to that in fresh kidneys. More recently they confirmed the findings using monoclonal antibodies to rat class I and II antigens. Therefore, in the broad sense of making a graft fit or suitable, adaptation does occur in enhanced grafts, but this seems to be best attributed to the loss of passenger cells.

It appears most likely that enhancement is maintained by an active regulation of the host response to the donor graft antigens, although the mechanisms underlying this regulation are unclear. While an anti-idiotypic response might assist in the induction phase of enhancement this mechanism could not be supported for the maintenance phase (Batchelor et al. 1977; Stuart et al. 1979; Morris, 1980). For example, circulating anti-idiotypic antibody could not be detected in recipients carrying long-surviving kidney allografts (Stuart et al. 1976). Serum-blocking factors likewise have been generally discounted as a mechanism. The rapid destruction of enhanced mouse skin allografts following the injection of rabbit complement alone, argues against the idea that a graft is protected by a coating of blocking antibody (Koene et al. 1973). Also no blocking activity has been found in the sera of long-surviving renal allo-

grafts using MLR and ^{51}Cr release (Stuart et al. 1979; Morris, 1980). The role of T cells in the suppression of the immune response has been widely reported in various models (reviewed by Tilney et al. 1979b) and will be further discussed in Section 1.14. Suppressor T cells have been implicated in adoptive transfer and in in vitro experiments where prior tolerance has been induced following ALS and tissue extract treatment (Kilshaw et al. 1975) or TLI (Strober et al. 1979; Myburgh et al. 1980a,b, 1983; Smit et al. 1980, 1981). The evidence of Dorsch and Roser (1977) suggests that neonatal tolerance is maintained in the adult rat by long lived recirculating suppressor T cells, and Stuart et al. (1976) were able to prolong renal allograft survival following the adoptive transfer of spleen cells from enhanced kidney recipients. Batchelor et al. (1977), on the other hand, were not able to demonstrate suppressor activity in enhanced renal allograft recipients. While a definitive evaluation of the many different mechanisms that contribute both to the induction and maintenance of enhancement is not possible, the role of T suppressor cells remains the most plausible explanation for the maintenance phase.

1.14 MECHANISMS OF TRANSPLANTATION TOLERANCE

One of the key questions in transplantation immunology is whether transplantation tolerance is induced and maintained by passive central clonal deletion or active peripheral suppressor mechanisms, or a combination of both. Classical neonatal transplantation tolerance was originally thought to be maintained by the deletion

of specific antigen reactive cells. This notion has not remained unchallenged, however, and is slowly being modified as evidence accummulates which supports the role of an active mechanism (Dorsch and Roser, 1977; Gruchalla and Streilein, 1982). Several mechanisms have been proposed besides clonal deletion, the main ones being serum-blocking factors, anti-idiotypic antibodies and T suppressor cells. These have already been noted in the above discussion on enhancement (Section 1.13.6) but, in this section, it is proposed to present some further evidence for these mechanisms. Because a very large body of evidence exists in support of these proposals, once again it is possible to give only a brief summary. For general reviews on mechanisms, see Brent et al. (1976), Elkins (1979) and Nossal (1983).

1.14.1. Clonal deletion

As already indicated, Billingham et al. (1956a) attributed neonatal tolerance to a central failure of the immune response, since the transfer of syngeneic lymphocytes could restore immunological reactivity - an idea consistent with Burnet's clonal selection hypothesis (Section 1.10). This idea essentially became the conventional view of transplantation tolerance.

However, the evidence from subsequent studies has not been unequivocal. Some studies where normal lymphoid cells have been used to abolish the tolerance state have given evidence of successful abolition of tolerance and hence interpreted as consistent with clonal deletion (Billingham et al. 1963), while others were not able to

abolish similar tolerance (Ramseier, 1973; Rieger and Hilgert, 1977; Hilgert, 1979; reviewed by Elkins, 1979). Similarly, some studies which looked at T cell function in neonatal tolerized animals using MLC, CML and GVH assays, supported the idea of donor-reactive lymphocytes having been deleted or inactivated in such animals (Wilson and Nowell, 1970; Atkins and Ford, 1972; Brooks, 1975; Gruchalla and Streilein, 1982), while others have demonstrated that residual cytotoxicity may persist in experimentally tolerant animals and in tetraparental chimeras (Hellstrom et al. 1971; Hellstrom and Hellstrom, 1974; Bansal et al. 1973; Droege and Mayer, 1975; Dorsch and Roser, 1977; Tutschka, et al. 1981a; Section 1.12). In studies in our own laboratory we have shown that adult tolerant animals respond normally to donor alloantigens in vitro (Chapter 6).

As a consequence of these and other investigations, the clonal deletion concept has been seriously challenged and there now seems to be a general consensus that unresponsiveness is maintained through active regulatory mechanisms rather than through the passive clonal deletion mechanism (Hilgert, 1979).

1.14.2. Serum blocking factors

Antibodies or immune complexes have been suggested as a mechanism of suppressing the immune response, either by the direct masking of potential activating determinants by specific antibodies in the serum, or by the presence of antigen-antibody complexes. Voisin et al. (1968, cited in Brent et al. 1976) raised the idea that humoral blocking factors inhibited immunological function in tol-

erant animals by enhancing tumour growth with serum from tolerant mice. Subsequently, Hellstrom and coworkers reported that serum from tolerant animals blocked killing of donor-type cells *in vitro* with evidence pointing to an antigen-antibody complex as being the blocking factor (Hellstrom *et al*. 1971; Bansal *et al*. 1973; reviewed by Hellstrom and Hellstrom, 1974). Similar findings were reported by Chutna *et al*. (1973). However, the animals used by these workers were not fully tolerant and Brent *et al*. (1972) who used completely tolerant mice could not confirm this. Other studies soon confirmed that blocking activity was detectable but only in sera from partially tolerant mice (Brooks, 1975).

Elkins (1973) using rat bone marrow chimeras was unable to find evidence for an active cellular or humoral suppressor mechanism and Hasek *et al*. (1975) could not show that blocking factors played any role in tolerance induction. More recently, other groups have failed to demonstrate the presence of blocking factors *in vitro* (Waterfield *et al*. 1981; Moran *et al*. 1983).

Controversy concerning the role of serum blocking factors has arisen from the different assay systems used (Law *et al*. 1974) and the relevance of *in vitro* observations to tolerance maintenance *in vivo* (Brent *et al*. 1976).

1.14.3. Anti-idiotypic antibodies

Ramseier (1973) and Rowley *et al*. (1973) suggested that anti-idiotypic antibodies may eliminate lymphocytes carrying receptors for the tolerated antigens in chimeric animals, and the work of Binz and Wigzell (1975),

suggested an important role for anti-idiotypic antibodies in tolerance maintenance. However, its true role in maintenance of transplantation tolerance remains speculative and, as already noted in Section 1.13.6.2, this mechanism could not be supported for enhancement studies. More recently, Moran et al. (1983) could find no evidence for an anti-idiotypic antibody suppression mechanism. According to Batchelor (1983), one of the problems in analyzing this mechanism has been the difficulty in setting up reproducible experimental systems.

1.14.4. T suppressor cells

The greatest body of evidence for an active mechanism has come from studies on suppressor function. Since the early work by Dorsch and her colleagues who described suppressor cell activity in neonatal tolerance and presented evidence for the involvement of T cells (Dorsch and Roser, 1974, 1975; reviewed by Roser and Dorsch, 1979), there has been a flood of reports, using both in vivo and in vitro assays in a number of different experimental models, all of which suggest the involvement of an active cellular suppressor mechanism in the maintenance of transplantation tolerance. Experimental systems include: neonatal tolerance induced by F_1 bone marrow cells in rats (Dorsch and Roser, 1977, 1982a,b; Hilgert, 1979); neonatal tolerance in mice (Hasek and Chutna, 1979; Streilein, 1979; Holan et al. 1980; Vegh et al. 1980; Janossy et al. 1983); adult tolerance (Section 1.12) induced by various methods such as bone marrow chimeras in lethally irradiated rats (Tutschka et al. 1981a,b, 1982, 1983); bone marrow transplants to

recipients of TLI (Strober et al. 1979; Myburgh et al. 1980a,b, 1983; Smit et al. 1980, 1981), ALS and tissue extracts (Kilshaw et al. 1975; Brent and Opara, 1979; Hilgert, 1979), blood pretreatment (Horsburgh et al. 1981; Maki et al. 1981; Marquet and Heystek, 1981; Lenhard et al. 1982), cyclosporine (Hutchinson et al. 1981; Hess et al. 1983), human kidney transplant patients maintained on immunosuppressive drugs (Charpentier et al. 1982), organ culture of islets and ALS treatment of recipient (Zitron et al. 1981b), and enhancing antibody (Stuart et al. 1976; Section 1.13).

However, not all investigators have been as successful. While some could not consistently demonstrate suppressor cell activity (Moran et al, 1983), others failed to detect any evidence at all for such activity (Elkins, 1973; Brooks, 1975; Batchelor et al. 1977; Weigle, 1978; Gruchalla and Streilein, 1982).

A number of studies have pursued the phenotype and functional aspects of the suppressor cells (reviewed by Roser and Dorsch, 1979; Roser et al. 1983). Studies in the rat by Dorsch's group have shown the suppressor cell distribution to reside within the lymphoid tissues of tolerant animals and suppression is mediated by long-lived, thymus-derived (Dorsch and Roser, 1977), rapidly recirculating (Dorsch and Roser, 1975) cells. More than 95% are T cells (Dorsch and Roser, 1977) as judged by several phenotype criteria. These cells carry the monoclonal W3/13 (Williams et al. 1977; Roser and Dorsch, 1979) and serologic pta (Roser and Dorsch, 1980) markers and lack surface immunogobulin and Fc receptors

(Dorsch and Roser, 1977). In the mouse these cells carry the Thy-1 marker (Hilgert, 1979), but the Lyt phenotype is not yet known (Roser et al. 1983). Some have reported results which implicate the existence of more than one suppressor cell type in tolerant mice (Gorczynski and Macrae, 1979).

In most studies, suppressor activity has been shown to be specific (Roser et al. 1983). Some have reported the development of a progression from alloantigen non-specific suppressor cells to specific suppressor cells after transplantation. Tutschka et al. (1981b) demonstrated the appearance of nonspecific suppressor cells in rat bone marrow chimeras at 40 days after grafting, being replaced by specific suppressor cells at 250 days. This sequence is similar to reports with recipients conditioned by TLI (Strober et al. 1979; Section 1.12.3).

Beyond identification of their presence in particular models, the precise mode of action of suppressor cells is not clear. Some have suggested that the effect of suppression might be to delete or inactivate clones of graft antigen reactive lymphocytes (Dorsch and Roser, 1977; Tutschka et al. 1981a). Tutschka's group found that, in their model, spleen cells harvested at various times after transplantation and tested both in vivo and in vitro, could no longer be detected by in vitro methods at 720 days post-transplantation (Tutschka et al. 1981b). More recently, Heuer et al. (1982), using a T suppressor cell clone originally isolated from mice rendered tolerant to BSA, suggested that clonal deletion could result from T suppressor cell activity.

While the majority of investigators it seems, favour an active T cell suppressor mechanism as being responsible for the maintenance of transplantation tolerance, some have questioned the significance of the suppressor mechanism. For example, much evidence for suppressor cell activity has come from adoptive transfer experiments of T cells to syngeneic recipients which are then test grafted. However, since it is usually necessary to partially immunosuppress recipients using sublethal irradiation or ALS (Kilshaw et al. 1975; Hasek and Chutna, 1979; Roser and Dorsch, 1980), the relative importance of suppressor cells has come under question (Batchelor, 1983). Furthermore, Hasek and Chutna (1979) have argued that if suppressor cells play a major role in tolerance induction, then a sublethal dose of irradiation should terminate the tolerance state. While Fefer and Nossal (1962) were able to abolish partial tolerance with doses of 350-450 rads of irradiation, Hasek's group failed to abolish tolerance in rats using 400 rads. This led them to suggest that suppressor cells may not be the only mechanism involved (Hasek and Chutna, 1979) - a conclusion arrived at recently by Gruchalla and Streilein (1981). Roser and Dorsch (1980) also failed to abolish suppression with irradiation (750 rads), although they could do so by combining irradiation with small doses of normal lymphoid cells. Classic in vitro suppression assays have also come under criticism because of the difficulty of interpretation in cell mixture experiments between genuine suppression and artifact due to culture conditions (Auchincloss and Sachs, 1983).

1.14.5. Conclusions

The mechanism of transplantation tolerance is still poorly understood some 30 years after the first experimental induction. Factors which have contributed to this confusion are the lack of universal criteria by which tolerance can be assessed and the use of many different experimental systems to induce adult tolerance.

Although clonal deletion has been a widely held and unifying concept in immunological tolerance, it has for some time come under challenge. The clonal selection concept has failed to account for the fact that there are present self-recognizing B and T lymphocytes in normal animals, and F_1 animals do respond to parental antigens (Streilein, 1979; Ebringer, 1981). Moreover, there is mounting evidence that other mechanisms are involved in achieving unresponsive or tolerant states. T suppressor cells, as an active mechanism in transplantation tolerance maintenance, has been increasingly demonstrated in tolerant animals during the past decade. Finally, the numerous studies suggest that transplantation tolerance is a complex phenomenon which possibly involves more than one mechanism in its maintenance (Brent et al. 1976; Charpentier et al. 1982).

CHAPTER 2

MATERIALS AND METHODS

2.1 EXPERIMENTAL ANIMALS

All animals used were inbred strains of mice obtained from the Animal Breeding Establishment at the John Curtin School of Medical Research.

Male BALB/c ($H\text{-}2^d$) mice, approximately 6-10 weeks old, were used as donors of pancreatic islets, thyroid tissue, peritoneal and spleen cells. Male CBA/H ($H\text{-}2^k$), 10-15 weeks old, were used as recipients of donor tissue. BALB/c foetuses at 17 days gestation were used as donors of foetal pancreatic tissue. Foetuses were obtained in the following way: BALB/c breeding pairs were set up and mating was monitored by daily inspection of female mice for the presence of a vaginal plug. Seventeen days after identification of the plug, pregnant mice were killed by cervical dislocation and the foetuses were removed by hysterectomy. Male C57BL10J ($H\text{-}2^b$) 8-10 weeks, were also used as a source of thyroid tissue in some experiments.

Experimental animals were housed either singly or in groups of up to 10 in wire topped cages containing normal litter, as well as cotton wool in the case of diabetic animals. Animals were provided with food and water ad libitum and the standard feed was Mecon rat and mouse cubes (Fidelity Feeds, Murrumburrah, N.S.W.).

2.2 TISSUE CULTURE MEDIA

RPMI 1640 medium (M.A. Bioproducts, USA) was prepared by dissolving 10.4gm of medium powder in 1 litre of double distilled, de-ionised water supplemented with 1.0gm of sodium bicarbonate.

Eagle's Minimal Essential Medium (EMEM) was prepared

by dissolving 10.0gm of medium powder (GIBCO) in 1 litre of double distilled, de-ionised water supplemented with 2.2gm sodium bicarbonate.

RPMI 1640 and EMEM were supplemented with antibiotics (penicillin, 100 units/ml; streptomycin, 100µg/ml; neomycin, 100µg/ml) and sterilized by filtration through a 0.22µ filter (Multipore Corporation). Every two weeks after the initial preparation, RPMI 1640 medium was supplemented with L-glutamine (0.3gm/100ml).

Heat inactivated foetal calf serum (HIFCS) was prepared by incubating each serum batch (Flow Laboratories, Australia) at 56°C for 30 minutes.

Hanks Balanced Salt Solution (HBSS) was prepared according to the procedure described by Hanks and Wallace (1949). HBSS was sterilized by autoclaving at 112°C for 20 minutes.

HEPES buffer (N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid) (Sigma) was prepared by adding 25gm HEPES powder in 100ml HBSS adjusted to pH 7.4 and sterilized by filtration.

Phosphate Buffered Saline (PBS; calcium and magnesium free, pH 7.3) was prepared according to the following formula: sodium chloride, 80gm; disodium hydrogen phosphate, 12.5gm; monosodium hydrogen phosphate, 4.0gm; distilled water, 1 litre. PBS was sterilized by autoclaving at 121°C for 20 minutes.

Normal saline (0.9% sodium chloride) was prepared by dissolving 9.0gm sodium chloride in 1 litre of distilled water and sterilized in the same way as PBS.

Bovine serum albumin (BSA) was prepared by dissolving

7.5gm of bovine albumin powder (Armour Pharmaceutical, England) in 50ml of HEPES-buffered HBSS supplemented with antibiotics. The solution was sterilized by filtration through a 0.22μ filter before use.

2.3 ISOLATION AND CULTURE OF TISSUE

2.3.1. Pancreatic islets

The isolation and culture of pancreatic islets has been summarized diagrammatically in Fig. 2.1. Pancreatic islets were prepared by a modification of the method previously used in our laboratory (Bowen et al. 1980). The pancreas from 4 BALB/c donors was removed and placed in a siliconized 20ml glass scintillation vial containing collagenase (Boehringer Mannheim) at a concentration of 1.5mg/ml, in 5ml HEPES-buffered HBSS supplemented with antibiotics (penicillin, 100units/ml; streptomycin, 100μg/ml; neomycin, 100μg/ml), BSA (1.5mg/ml) and deoxyribonuclease (DNase I: Sigma)(0.01mg/ml). The collagenase and DNase had been filtered through a 0.22μ disposable filter. The tissue was then digested for 20 minutes using a water bath set at 37°C, with a mechanical shaker. The enzymatic digestion was arrested by the addition of 10-15ml of chilled HEPES-HBSS solution. The digest was allowed to settle on ice for 5 minutes before removing the supernatant. Five ml of half strength collagenase (0.75mg/ml) was added and a second digestion (5 minutes) was carried out. This short digestion was repeated 3 times. After the final digestion, individual islets were identified by using a stereomicroscope (Olympus SZ-111, Japan) set at 16x magnification. Islets were picked using a sili-

DONOR

BALB/c ($H\text{-}2^d$)

PANCREAS REMOVED FROM 4 DONORS

RECIPIENT

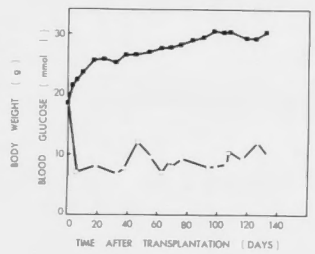
CBA ($H\text{-}2^k$)

MADE DIABETIC WITH STREPTOZOTOCIN INJECTION

TRANSPLANTATION

7 CLUSTERS UNDER KIDNEY CAPSULE

GRAFT FUNCTION



COLLAGENASE DIGESTION

SHAKER WATER BATH 37°

1st DIGESTION (20 min)

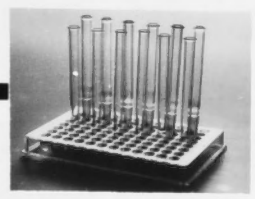
REPEAT 4 TIMES USING HALF STRENGTH COLLAGENASE (5 min. each)

ISLETS PICKED

STEREOMICROSCOPE (x 16)

REPICKED 3-4 TIMES

96 WELL U BOTTOM MICROTITRATION PLATE 50 ISLETS / WELL



CULTURE

GAS INCUBATOR 95% O_2 5% CO_2

INITIAL CULTURE 3 DAYS

REGAS AND CULTURE 4 DAYS

CLUSTERS IN 1008 DISHES

FIGURE 2.1 DIAGRAMMATIC REPRESENTATION OF PREPARATION, CULTURE AND TRANSPLANTATION OF MOUSE PANCREATIC ISLETS

conized narrow bore pipette attached to a rubber tube for mouth suction, and transferred to a petri dish containing isolation medium. Islets were repicked 3 to 4 times to eliminate exocrine tissue contamination. This method gave a relatively high yield of approximately 80-100 islets per mouse pancreas.

During the final pick, groups of 50 islets were transferred to siliconized 10ml glass centrifuge tubes containing 2ml RPMI 1640 culture medium supplemented with 10% HIFCS and allowed to settle on ice for 10 minutes. Most of the supernatant was then removed leaving 0.3-0.4ml. Using a siliconized Pasteur pipette this fluid was gently churned to resuspend the islets and then taken up and transferred to hydrophobic U-shaped wells in a 96 well microtitration plate (Linbro/Titertek, Cat. No. 76-212-05, Flow Laboratories, USA). After allowing the islets to settle for 5 minutes on ice, as much as possible of the medium was removed from each well, while taking care not to pick up islets and replaced with 100µl of fresh culture medium. Islets were then aggregated by centrifugation (200g) for 4 minutes using a TJ6 (Beckman) centrifuge. A later improvement involved the use of glass, bottomless tubes which were specifically made to fit firmly over the wells of the microtitration plate. In this way, groups of about 50 islets were transferred directly to the plate and allowed to settle. The supernatant was then removed, the glass tubes taken off and the plate centrifuged as before. After centrifugation, the isolation medium was removed and replaced with culture medium (100µl per well) as before and re-centrifuged. The microtitration plate was then placed

in a humidified portable incubator and charged for 10 minutes with a 95% O_2 and 5% CO_2 gas mixture. The islets remained in this atmosphere for 3 days during which time they formed into intact clusters. After 3 days, islet clusters were removed from the wells and each placed in a separate 35mm hydrophobic petri dish (Falcon 1008) containing 0.75ml of fresh culture medium. The culture medium was subsequently changed every 2 days until day 7 when the islets were ready for transplantation. Depending on the availability of sufficient tissue for one transplant (350 islets), tissue could be left in culture for as long as 9 days. The incubator was regased after each medium change and the ambient temperature was maintained at 37°C throughout the organ culture period.

2.3.2. Foetal pancreas

This procedure has been described by Simeonovic and Lafferty (1981). Briefly, BALB/c foetuses at 17 days gestation were killed by decapitation and the pancreas removed intact from each foetus and transferred to RPMI 1640 culture medium supplemented with 10% HIFCS. To facilitate gas diffusion, each pancreas was subsequently cut into 3 segments, transferred to a 35mm hydrophobic petri dish (Falcon 1008) containing 1ml of culture medium and cultured in 95% O_2 and 5% CO_2 at 37°C for 10 days. Culture medium was changed 3 times per week.

2.3.3. Thyroid tissue

Thyroid lobes for culture were removed under sterile conditions from BALB/c donor mice which had been pretreated with cyclophosphamide (Endoxan-Asta, Bristol Laboratories, Australia). Cyclophosphamide powder was first prepared

for injection by diluting it with distilled water to a final concentration of 20mg/ml. Two intraperitoneal injections were given at a dose rate of 300mg/kg body weight on days -4 and -2. Mice were killed on day 0 by chloroform inhalation.

For removal of the thyroid, the neck region was swabbed with 70% ethanol and a 2cm midline incission was made using a large scalpel blade (number 22, Swann-Morton, England). Using blunt dissection, the underlying salivary glands were parted and the muscle sheath around the trachea was split longitudinally and separated. This allowed identification of the two thyroid lobes, one on either side of the trachea. The lobes were removed as intact as possible and placed initially in HBSS supplemented with 0.013% w/v sodium bicarbonate and antibiotics. When all thyroids had been removed, two lobes were placed in each hydrophobic 35mm petri dish (Falcon 1008) containing 1ml of EMEM supplemented with 10% HIFCS. Thyroid tissue was cultured in 95% O_2 and 5% CO_2 at 37°C for 21 days, during which time the culture medium was changed 3 times per week.

2.4 ANAESTHESIA

General anaesthesia was induced by peritoneal injection of avertin anaesthetic solution. Avertin was prepared by dissolving 1.0gm of 2,2,2-tribrom-aethanol (Fluka AG, Switzerland) in 1.0ml of 2-methyl-2-butanol (Fluka AG, Switzerland). The solution was diluted to a final volume of 50ml with hot tap water (50°C) and shaken vigorously to mix. The solution was then decanted

at a separate site but close together, under the kidney capsule. When the capsule was released the graft was held securely against the kidney. During the transplant procedure the kidney was swabbed regularly with HBSS to prevent dehydration. At the conclusion of the transplantation procedure, the kidney was replaced into the abdominal cavity and the wound closed with 3 to 4 autoclips (9mm; Clay Adams, USA). The transplanted animal was then placed under an incandescent lamp until fully revived. Recipients of islet tissue, in most cases received 7 clusters or approximately 350 islets.

2.6 INDUCTION OF DIABETES AND MANAGEMENT OF DIABETIC MICE

2.6.1. Preparation of acetate buffer

A stock solution of acetate buffer (pH 4.4) was prepared according to the following formula: 30.5ml 0.2M acetic acid, 19.5ml 0.2M sodium acetate, 0.9gm sodium chloride. The solution was made up to a final volume of 100 ml with distilled water.

2.6.2. Induction of diabetes

Diabetes was induced in CBA recipients of islet allografts by using a single tail vein injection of streptozotocin (Calbiochem-Behring, La Jolla, California). Immediately before injection, 30mg of streptozotocin was dissolved in 1.0ml acetate buffer (Section 2.6.1). This solution was drawn up in a 1.0ml syringe and injected using a 26 guage needle, at a dose of 300mg/kg body weight.

2.6.3. Management of diabetic mice

To control dehydration, mice were given intraperitoneally 1.0ml of warmed (37°C) normal saline per gram of lost body weight from days 3 to 6 (inclusive) post-streptozotocin injection. In addition, mice were maintained with a single, daily subcutaneous injection of 0.8 units of Ultralente insulin (CSL) from day 3 to 14 post-streptozotocin. At day 17 and again just before transplantation, blood and urine glucose levels were determined (Section 2.8, 2.9). Animals which showed a consistent non-fasting blood glucose reading of 20 ≥mmoles/litre were used as recipients of islet allografts. Diabetic mice were transplanted 17 to 35 days after streptozotocin treatment.

2.7 THYROIDECTOMY OF THYROID ALLOGRAFT RECIPIENTS

Recipients of cultured thyroid allografts were partially thyroidectomized 3 days before thyroid transplantation. This procedure facilitates the assay of graft function (Section 2.11) and stimulates the production of thyroid-stimulating hormone thereby promoting the growth of grafted thyroid tissue (Lafferty and Cunningham, 1975). The procedure used for removal of recipient thyroid tissue was the same as for the removal of donor thyroid tissue, except that animals for thyroidectomy were placed under anaesthesia (Section 2.4) and care had to be taken to avoid damage to local blood vessels and particularly to the recurrent laryngeal nerve which runs close to the left thyroid lobe. As much as possible of each thyroid lobe was excised by blunt dissection. The muscle sheath and salivary glands were then put in place and the incision closed with 2 or 3 autoclips. The mice were then placed

under an incandescent lamp until they recovered from the anaesthesia.

2.8 BLOOD GLUCOSE MEASUREMENTS

Non-fasting blood glucose levels were determined in diabetic mice and recipients of islet allografts, using a Glucose Analyzer 2 (Beckman, USA). Whole blood (10µl) was collected from the tip of the tail using a 10µl microcap (Drummond, USA) and transferred immediately to a microcentrifuge tube (Vetri, 1.5ml with attached cap; Max Richter, West Germany) containing 40µl of 0.66M perchloric acid for blood deproteinization. The microcentrifuge tube was first vortexed to mix and then centrifuged for 90 seconds in a microcentrifuge (Beckman Microfuge). Two 10µl aliquots were then taken from each supernatant and the glucose content determined against a glucose standard. Duplicate assays were averaged and measurements corrected for sample dilution and expressed in mmoles of glucose/litre. The lower limit of sensitivity for the analyzer was 0.56 mmoles glucose/litre. The normal range for male CBA mice is given as the mean $\pm$ 1.96 standard deviations. It was determined from several groups of normal, non-fasting male CBA mice (170 animals) which had been bled from the tail vein during early to mid afternoon. Data were pooled and the 95% confidence interval for blood glucose levels was calculated. The normal range for non-fasting animals was determined as 8.5 $\pm$ 2.32 mmoles glucose/litre.

2.9 URINE GLUCOSE MEASUREMENTS

As with blood glucose measurements, urine glucose measurements were determined in animals which were diabetic or carried an islet allograft. Individual mice were placed in a round cage constructed with a wire mesh bottom. Urine was collected over a 24 hour period by means of a funnel which spanned the cage floor and drained into a 50ml cylinder. During this time, food and water were provided ad libitum. At 24 hours the volume or urine was noted and and an aliquot (100µl) of urine was diluted in 10ml distilled water. If no urine was visible due to evaporation, the collection cylinder was rinsed with 2ml of distilled water (reconstituted volume) and a sample was removed for glucose determination. A 400µl sample of the dilution was transferred to a microcentrifuge tube and centrifuged for 90 seconds to remove any debris. Two 10µl aliquots were then taken from each supernatant and the glucose content assayed using the method described in Section 2.8. Measurements were corrected for sample dilution, and the amount of glucose excreted in 24 hours was calculated according to the following formula:

$$\text{Urine glucose output (mmoles/24hrs)} = \text{24hr urine volume or reconstituted volume (ml)} \times \frac{\text{mmoles glucose/litre}}{1000}$$

Normal or diabetes-reversed CBA mice failed to excrete detectable amounts of glucose.

2.10 NEPHRECTOMY

Animals carrying functional islet allografts had their graft removed by nephrectomy at the conclusion of the experiment. The kidney was exposed as described in Section 2.5 above so that the main renal artery and vein were visible. These blood vessels were then ligated with sterile surgical silk (size 000, Ethicon, USA). The kidney was cut free and the excision site swabbed with HBSS. The incised skin was sutured with autoclips. After examination, the removed kidney was fixed in 10% formol saline for histology (Section 2.12). Non-fasting blood glucose levels were thereafter measured daily for one week. A return to the diabetic condition was an indication that the allograft had been functional and responsible for the maintenance of normoglycaemia. Nephrectomized animals usually returned to the diabetic condition within 1 - 3 days following the removal of the islet allograft.

2.11 MEASUREMENT OF THYROID FUNCTION

Thyroid function was followed by measuring the level of ^{125}Iodine (^{125}I) uptake by transplanted thyroid tissue. Details of this assay have been descibred by Lafferty et al. (1976a). Briefly, transplant recipients received an intraperitoneal injection of 15μCi of ^{125}I (code IMS 30, Amersham, England) at 21 days post transplantation and at 2 to 3 week intervals thereafter. Three days after the ^{125}I injection, animals were anaesthetized and the level of radiation emitted from the thyroid transplant was measured by positioning a scintillation probe above the site of the grafted kidney. Grafts emitting

more than 171 counts per minute (c.p.m.) were scored as functional. This figure is 2 standard deviations above the mean value obtained for known rejected allografts (Table 2.1).

2.12 HISTOLOGY

2.12.1. Preparation of formol saline

Formol saline was prepared according to the following formula: 100ml formaldehyde; 8.5gm sodium chloride; 4.0gm monosodium hydrogen phosphate; 6.5gm disodium hydrogen phosphate. The solution was made up to 1 litre with distilled water.

2.12.2. Preparation of tissue for histological examination

All allografted tissue was evaluated at the conclusion of the experiment by histological examination. The kidney carrying the graft was fixed in 10% formol saline for at least 3 days before trimming the tissue for histology. Paraffin sections were cut and stained with haematoxylin and eosin or with aldehyde fuchsin. The latter selectively stains the insulin containing granules of the beta cells of pancreatic islets (Bussolati and Bassa, 1974). Thyroid tissue sections were stained only with haematoxylin and eosin. Tissue sections were examined to determine the extent of damage to the graft and mononuclear cell infiltration in and around the transplanted tissue.

2.13 PREPARATION OF SPLEEN CELLS

BALB/c spleens were removed aseptically and placed in HBSS buffered with 0.013% w/v sodium bicarbonate. Spleens were then pressed through a fine stainless steel sieve into fresh HBSS. The resultant suspension was trans-

Table 2.1

^{125}I uptake by uncultured BALB/c thyroid allografts two weeks* after transplantation under the renal capsule of thyroidectomized CBA recipient mice

CBA Recipient	^{125}I uptake (mean cpm±SD)		
	Background	Graft	Net Graft
1	127±11	225±10	98±10
2	130±6	183±10	53±10
3	136±15	284±15	148±15
4	126±12	241±24	115±24
5	127±16	252±15	126±15
6	132±11	171±13	39±13
7	124±9	257±15	133±15
8	142±8	233±11	91±11
9	146±17	229±14	83±14
		Mean value ± SD :	98±36

* The day following the ^{125}I assay all grafts were removed. All showed only scar tissue remaining.

ferred to a centrifuge tube and allowed to settle on ice for 5 minutes to remove cell clumps. The supernatant was transferred to a sterile centrifuge tube and centrifuged at 500g for 5 minutes. The cell pellet was resuspended in HBSS and spleen cells (SC) counted in a haemocytometer using white blood cell counting fluid or, where viable SC were required, viability was determined by trypan blue exclusion. The cell density was then adjusted to the required level.

2.14 ULTRAVIOLET IRRADIATION OF SPLEEN CELLS

An ultraviolet (UV) meter (Black-Ray) was set up on an adjustable stand beneath the UV source (Philips Oliphant germicidal G 3DT8 lamp) in a laminar flow hood, at intensity 960$\mu W/cm^2$ in the 230 to 270nm range (Talmage et al. 1977). The spleen cell suspension (up to 2ml) was then placed in a coverless 35mm glass petri dish on the stand beneath the UV source and irradiated for 4 minutes. The suspension was swirled several times during irradiation to ensure complete irradiation of cells.

2.15 PREPARATION OF PERITONEAL CELLS

Donor mice were killed by cervical dislocation and their abdomens swabbed with 70% ethanol. An incision was made in the skin of the abdominal wall and the skin gently stripped back to expose the peritoneum. The peritoneal cavity was then irrigated with 5ml HEPES-buffered HBSS. The resulting cell suspension was withdrawn by syringe and centrifuged at 800g for 5 minutes. The cell pellet was resuspended in 1ml HEPES-buffered HBSS and cell viability determined using the trypan blue exclusion test.

The density of viable cells was assessed using a haemocytometer.

2.16 PREPARATION AND INJECTION OF CYCLOSPORINE (CyA)

CyA (a gift from Sandoz, Switzerland) was dissolved at 25mg/ml in olive oil using a stirrer within a hot water bath (60°C) for 20 mins. Recipients of CyA treatment were injected subcutaneously at a dose rate of 75mg/kg body weight beginning at day -1 from the injection of sensitised cells, to day 7 post-injection of cells.

2.17 ANTISERA

2.17.1. CBA anti-BALB/c antiserum

This antiserum was produced by immunizing 8 to 10 week old male CBA mice with multiple intraperitoneal injections of BALB/c spleen cells or spleen and thymus cells. A total of four weekly immunizations were given at a dose of 0.25 donor spleen cells and approximately 6.0×10^7 thymocytes per recipient. Animals were bled up to one week following the final injection. Antiserum titres of about 1/4000 were demonstrated by haemolysis assay (Parr and Oei, 1973) with rabbit complement (Section 2.18).

2.17.2. Monoclonal antisera and treatment of sensitised spleen cells

A monoclonal anti-Lyt 2.1 ascitic fluid was obtained from Australian Monoclonal Development (Sydney); monoclonal anti-Thy 1.2 was obtained from Dr I. Weissman. Normal rabbit serum which had been absorbed on outbred mouse spleen and tested to ensure low toxicity (Section 2.18) was used as a source of complement.

Sensitised spleen cells (Section 2.20) at a concentration of $2x10^7$/ml were incubated with a final antibody concentration of 1/10 (anti-Thy 1.2) or 1/100 (anti-Lyt 2.1) in serum free HBSS for 30 minutes on ice. The cells were then washed and resuspended in a predetermined dilution of rabbit serum and incubated at 37°C for 30 minutes. Cells were washed once more, suspended in HBSS and counted. In different experiments, 33 to 52% of spleen cells were killed by anti-Thy 1.2 treatment and 23 to 34% by anti-Lyt 2.1 treatment. Less than 5% of spleen cells were killed by complement alone. Five $x\ 10^7$ untreated spleen cells or remaining cells after treatment with complement alone or antibody and complement, were injected intravenously into recipients.

The specificity of the two antibodies was tested by measuring Concanavalin A (ConA)-induced Interleukin-2 (IL-2) production from ConA spleen cell blasts (Andrus et al. 1981) treated as described above. Anti-Thy 1.2 and complement treatment completely abolished the ability of the blasts to produce IL-2 whereas anti-Lyt 2.1 and complement treatment did not affect the ability of the blasts to produce IL-2.

2.18 PREPARATION OF COMPLEMENT

Normal rabbit serum was used as a source of complement. Young adult rabbits were anaesthetized with an intravenous injection of 1.5ml Nembutal (sodium pentobarbitone: Abbot Laboratories, Australia) and bled out via a cardiac puncture. The collected blood was placed in a 37°C room for 1 hour to promote clot formation, after which the

clotted blood was rimmed and placed on ice for 1 hour before collecting the serum in a precooled centrifuge (1380g for 10 minutes). Rabbit serum was absorbed for 30 minutes on outbred mouse spleens and then removed and stored in 0.5 to 1.0ml aliquots at -80°C. Complement was tested using a standard chromium release assay (Section 2.23.3)

2.19 CYTOTOXIC ANTIBODY ASSAY

Cytotoxic antibody titrations were carried out using either trypan exclusion as an indicator of cell viability (Carlson and Terres, 1976) or a standard chromium release assay (Section 2.23.3) using ^{51}Cr labelled BALB/c cells as targets.

2.20 PRODUCTION OF SENSITISED SPLEEN CELLS

Male CBA mice were immunized *in vivo* by the intraperitoneal injection of 2.5×10^7 P815 or EL-4 cells grown from tissue culture (Section 2.22). Spleen cells were prepared from the mice 10 to 12 days later and tested for cytotoxic activity (Section 2.23.3) on P815 or EL-4 before injection (intravenous) into recipients. While waiting for the determination of cytotoxic activity, spleen cells were maintained in isolation medium on ice for about 5 hours.

In vitro sensitisation was achieved by culturing CBA lymph node cells with BALB/c spleen cells in 1ml cultures in 24 well trays (Linbro 76-033-05) using EMEM. One million CBA lymph node cells were cultured with 2×10^6 BALB/c spleen cells. The spleen cells had previously been cultured with mitomycin C (Sigma) at a concentration of 35µg/ml in EMEM for 90min at 37°C in 5% CO_2 in air. After 3 washes in EMEM they were plated with the responder lymph node cells. The cultures were

held at 37° in 5% CO_2 in air for 4 days. Expanded populations of activated T cells were prepared essentially as described by Woolnough and Lafferty (1979). The cells were subcultured on day 4 at 10^5/ml in EMEM containing spleen cell derived Interleukin 2 (IL-2) at a concentration predetermined to give optimal T cell growth (Lafferty et al. 1980). Cells were cultivated in flasks at 37° in 5% CO_2 in air for a further 3 days. At this time the expanded populations were 99% viable and had high cytotoxic potential.

2.21 PRODUCTION OF CONCANAVALIN A- ACTIVATED SPLEEN CELL SUPERNATANTS (CS)

The method for CS production has been described by Andrus and Lafferty (1980). Spleen cells were suspended in serum-free EMEM supplemented with 0.1mM 2-mercaptoethanol (2ME, Sigma) and antibiotics. Five ml aliquots were plated into Falcon 3013 culture dishes and pulsed with 5µg/ml ConA (Sigma) for 2 hrs at 37°C. The cell monolayers were then washed with HBSS to remove unbound ConA, and incubated in 10ml fresh serum-free culture medium for a further 16-20 hours at 37°. The supernatant was harvested, concentrated 10-fold through a Minicon macrosolute concentrator (B-15, Amicon, Lexington, Mass.), sterile filtered through a 0.22µm filter and stored at -20°C.

2.22 TUMOUR CELL LINES

The tumour cell lines, P815, a mastocytoma from the DBA2 ($H\text{-}2^d$) mouse strain, and EL-4, a T cell lymphoma from the C57BL/6J strain ($H\text{-}2^b$), were maintained by passage in tissue culture every 3 or 4 days. Cells were maintained in 25 cm^2 tissue culture flasks (Lux Scientific Corp., California) containing 5ml EMEM supplemented with 10% v/v HIFCS. The cells were incubated at 37°C in a humidified atmosphere of 10% CO_2 in air.

2.23 IN VITRO TEST OF IMMUNE REACTIVITY

Pooled axillary and mesenteric lymph node cells (LNC) from normal control or allografted CBA mice were tested for their capacity to respond to donor type alloantigen stimulation in vitro.

2.23.1. Mixed lymphocyte cultures (MLC)

Stimulator BALB/c SC at 3 x 10^6 cells/ml were prepared (Section 2.13) and incubated with mitomycin C at 30µg/ml of cell suspension at 37°C for 90 minutes in 10% CO_2 in air. mitomycin C was prepared by dissolving 2mg mitomycin C in 2ml normal saline and filtered through a 0.22µ disposable filter. Two x 10^6 LNC responders were mixed with 3 x 10^6 SC stimulators in a total volume of 2ml EMEM containing 10% v/v HIFCS and 0.1mM 2 ME. Cultures were set up in 24 well trays (Linbro) and incubated at 37°C in a humidified atmosphere of 10% CO_2 in air for 5 days.

2.23.2 Preparation of tumour cell targets

Lymphocyte preparations were tested for their cytotoxic activity against ^{51}Cr-labelled target cells. P815 or EL-4 suspensions were centrifuged (500g) for 5 minutes and resuspended at a concentration of 5×10^6 viable cells/ml in RPMI 1640 supplemented with 5% v/v HIFCS. The cells were labelled by the addition of $Na_2\ ^{51}Cr\ O_4$ (Amersham) to give a final concentration of 250 µCi/ml. Following incubation for 1 hour at 37°C in 5% CO_2 and air, unbound ^{51}Cr was removed by washing the cells 3 times in RPMI 1640 and then resuspended at a concentration of 10^5 viable cells/ml in EMEM supplemented with 10% v/v HIFCS.

2.23.3. Cytotoxicity assay

Using 96 U shaped well microtitration plates (Linbro/Titertek), 100µl of labelled targets were mixed with 100µl effector cells either in duplicate or quadruplicate and incubated for 4 hours at 37°C in a humidified atmosphere of 5% CO_2 and air. Spontaneous lysis of the targets was determined by mixing 100µl of targets with 100µl of EMEM plus 10% v/v HIFCS in quadruplicate microtitre wells. Total releasable label was determined by mixing 100µl of targets with 900µl distilled water in quadruplicate plastic centrifuge tubes (water lysis). Following incubation, the microtitre plate was centrifuged (500g for 1 minute) and 100µl supernatant was removed from each well without disturbing the cells. The water lysis tubes were vortexed, centrifuged (500g for 5 minutes) and 500µl supernatant was removed. Supernatants were counted for 10 minutes in a Packard Auto-Gamma 500 counter. Spontaneous release was generally 10 to 15% of the maximum release.

Cytotoxic activity is expressed in terms of $\log_{10}$ cytotoxic units (C.U.) per culture well, where one C.U. is defined as the activity required to lyse one target cell. The derivation of the cytotoxic unit has been described in detail elsewhere (Lafferty et al. 1976a; Woolnough and Lafferty, 1979).

CHAPTER 3

RESISTANCE OF ESTABLISHED ISLET AND THYROID ALLOGRAFTS TO ANTIBODY AND COMPLEMENT

3.1 INTRODUCTION

As discussed in Section 1.5, there has been considerable interest shown in the past over the respective roles of cell mediated and antibody mediated immunity in allograft rejection. Although graft rejection was considered in the 1950's to be primarily a cell mediated process (Mitchison, 1954; Snell, 1957; Brent, 1958), it is now evident that, depending on a number of factors, both humoral and cellular mechanisms may be involved.

With the demonstration of hyperacute rejection of kidney allografts in man (Kissmeyer-Nielsen et al. 1966; Williams et al. 1968), and various other tissues and organs in animal models by using the passive transfer of anti-donor antibody and complement (French, 1972; Koene et al. 1973; McDowall et al. 1973; McKenzie and Henning, 1978; Section 1.5.1.2), it is clear that antibody can contribute significantly to the rejection process. In particular, the evidence indicates that the antibody mediated attack is targeted on the vascular endothelium of the graft (Williams et al. 1968; Winn et al. 1973; Hume, 1974; Bogman et al. 1980).

That vascular endothelium and its possible replacement may be an important factor regulating whether or not a graft is rejected, has long been suggested. For example, the replacement of graft endothelium with host cells has been proposed as a possible mechanism for graft adaptation (Woodruff, 1959; Billingham, 1971; Williams et al. 1971; Section 1.13.6.2). Graft adaptation is a phenomenon based on a number of observations which have as their basis the fact that where an allograft is initially pro-

tected in some way and for a certain time from the host's immune reaction, the graft then appears to have "adapted" or overcome the "critical period" and, as a consequence, becomes less vulnerable to attack by the host (Woodruff and Woodruff, 1950; Maumenee, 1951; Woodruff, 1952, 1954; Weber et al. 1954; Cannon, 1957; Hart et al. 1980). Billingham et al. (1956a) and Medawar (1965) also suggested that the vascular endothelium of long tolerated grafts may have been partly replaced by cells of host origin. However, it was with the studies of the pathology of hyperacute rejection that the endothelium was implicated as the target of attack. Any repopulation of the graft vasculature by the host, therefore, could have important implications.

Organ culture in an oxygen-rich atmosphere not only reduces the immunogenicity of a tissue allograft (Lafferty et al. 1975; Sollinger and Bach, 1976; Talmage et al. 1976; Kedinger et al. 1977; Lafferty and Woolnough, 1977) but also destroys vascular endothelium in pancreatic islet and thyroid tissue (Moskalewski, 1965; Andersson and Hellerstrom, 1972; Lafferty and Woolnough, 1977; Parr et al. 1980a). When the cultured tissue is transplanted, revascularization with host vascular endothelium occurs. The cultured tissues, however, retain recognizable antigen and can be rejected when the recipient is challenged with donor strain leucocytes, either at the time of transplantation (Lafferty et al. 1976a; Simeonovic et al. 1980) or after the graft has become well established in the recipient (Lacy et al. 1979c; Bowen et al. 1981).

In this chapter we examined the effect of passively transferred alloantibody and antibody plus complement on the function of pancreatic islet and thyroid allografts.

Figure 3.1

Blood glucose levels (O) and body weight (●) of four CBA mice transplanted with 350 BALB/c pancreatic islets. All animals were injected with donor alloantibody (Ab) and antibody plus complement (Ab+C). Neither treatment had any effect on graft function. The normal range for blood glucose for CBA male mice (mean $\pm$ 2 S.D.) is indicated by the hatched area.

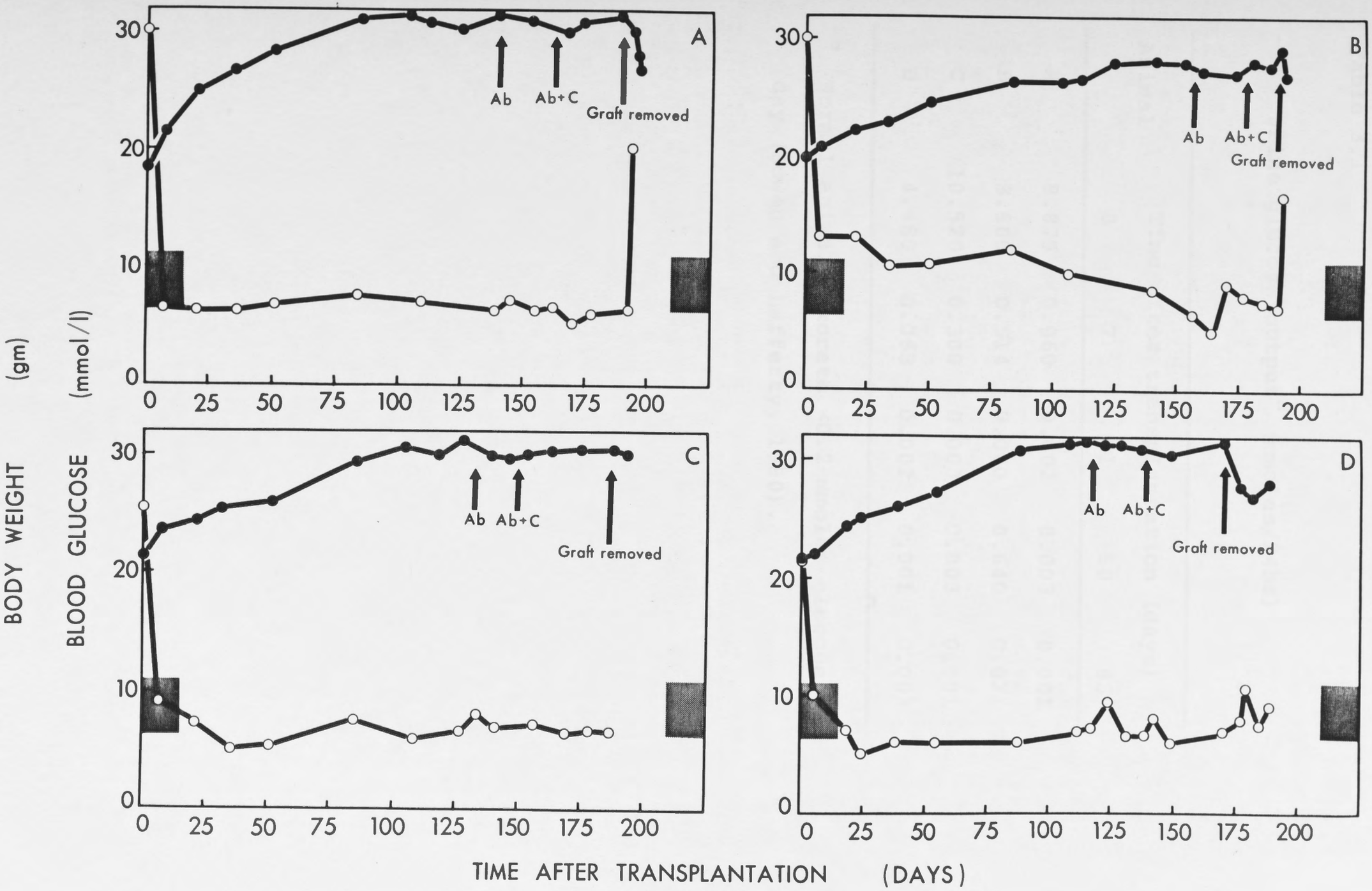

A
B
C
D
Ab
Ab+C
Graft removed
30
20
10
0
0 25 50 75 100 125 150 175 200
(gm)
(mmol/l)
BODY WEIGHT
BLOOD GLUCOSE
TIME AFTER TRANSPLANTATION (DAYS)

Table 3.1

Urine glucose output[a] (mmoles/24hr)

Animal	Time after transplantation (days)				
	0	7	21	50	85
A	8.875	0.060	0.002	0.003	0.001
B	3.600	0.514	0.080	0.640	0.021
C	10.520	0.300	0.003	0.003	0.001
D	4.480	0.063	0.002	0.001	0.003

[a] Normal animals excrete <0.2 mmoles glucose/ day (Bowen and Lafferty, 1980).

Table 3.2

Cytotoxic antibody titres in islet allograft recipients

Animal	Time after antibody injection (hr, hours; d, days)	Antibody titre
A	4 hr	1:5120
	4 d	1:1352
	7 d	1:5120
B	4 hr	1:2048
	4 d	1:640
	7 d	1:640
C	4 hr	1:2048
	4 d	1:2560
D	4 hr	1:3379
	4 d	1:5120

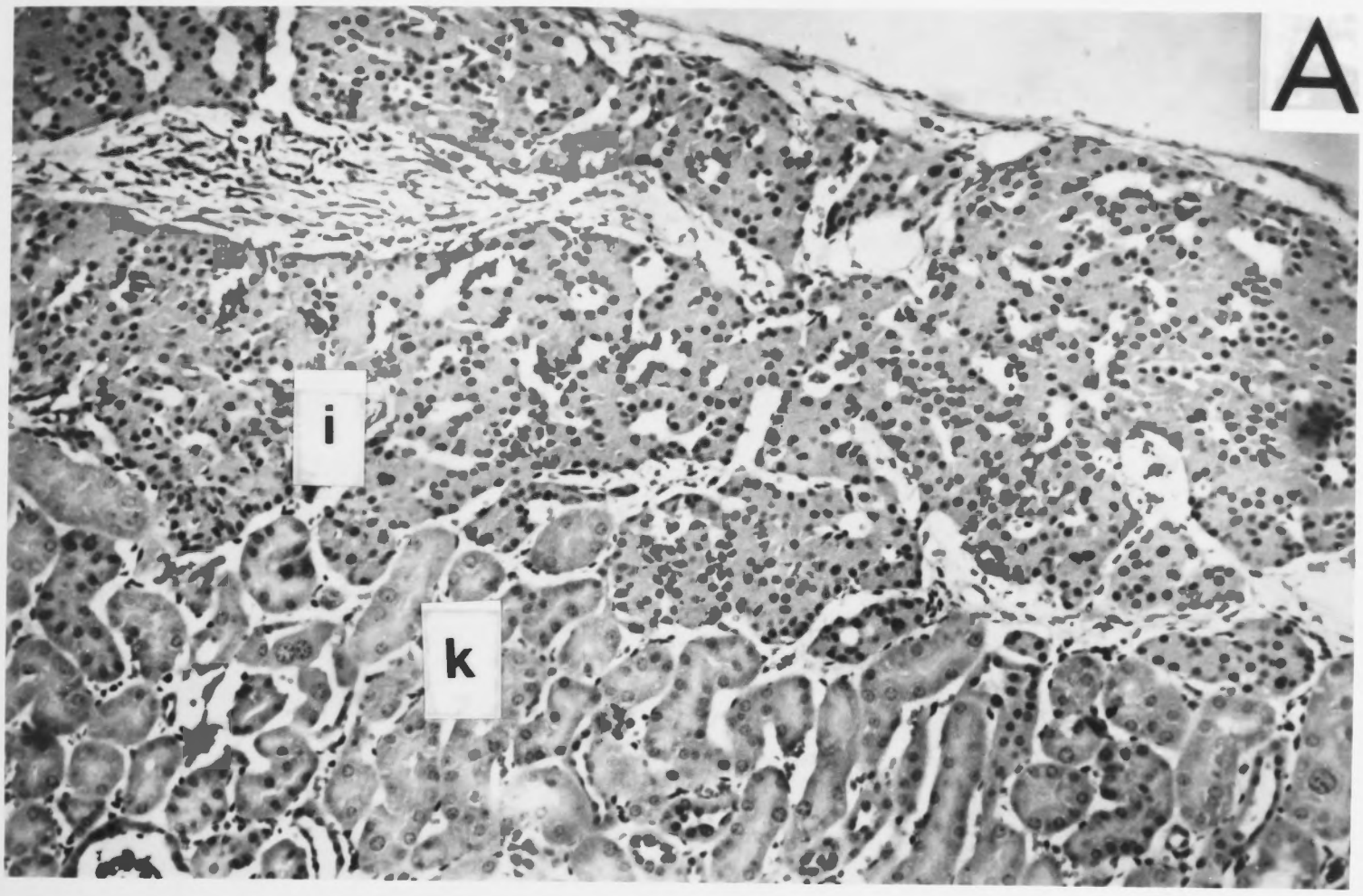
as a source of complement. The source and amount of complement was similar to that used by McKenzie and Henning (1978) to reject skin allografts in mice. Again, none of the four animals showed any functional evidence of rejection Fig. 3.1). When allografts were removed by nephrectomy about one month after the last injection, 2 animals returned rapidly to the diabetic condition (Fig. 3.1 A,B). One animal (Fig. 3.1C) died due to technical reasons associated with the nephrectomy, but histology of its pancreas did not reveal the presence of beta cells. It was assumed, therefore, that the graft in this animal, as with the other two, had been functional and responsible for the maintenance of normoglycaemia. The fourth animal (Fig. 3.1D) did not return to the diabetic state, suggesting that this animal's pancreas had recovered from the streptozotocin induced damage. The histology of its pancreas, which showed a number of light to medium stained granulated beta cells when compared to normal pancreas, confirmed this diabetes reversal. It should be noted that this is only the second out of many hundreds of such experimental animals investigated in our laboratory which failed to revert to the diabetic condition following the removal of the islet graft.

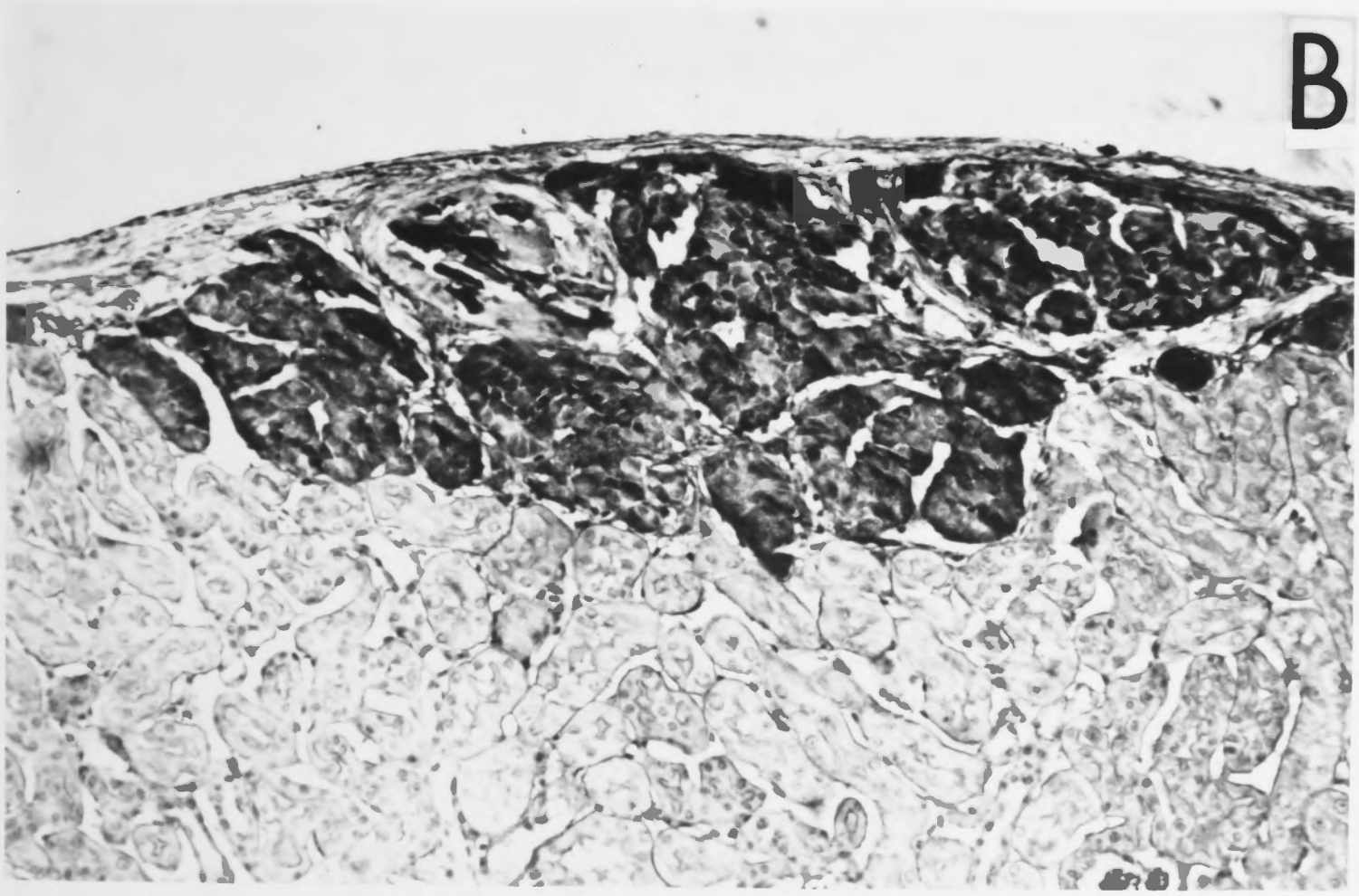
Histological examination of all the islet allografts showed that the tissue was normal and using aldehyde fuchsin staining, contained granulated beta cells (Fig. 3.2).

Figure 3.2

Histological appearance of islet allografts removed 35 days after injection of anti-donor alloantibody and complement. A. Note the absence of mononuclear cell infiltration and lack of any damage to islets (i, islets; k, kidney parenchyma) (haematoxylin and eosin; x 164). B. Note the dark staining granulated beta cells present in the transplanted tissue (aldehyde fuchsin; x 164).

(Note that these sections are representative of those taken from all experimental animals)


A
i
k


B

3.2.2. Resistance of thyroid allografts to rejection by antibody and complement

Eight male, thyroidectomized CBA mice were transplanted with BALB/c cultured thyroid. Four weeks after transplantation the iodine uptake was assayed and all grafts were shown to be functional (Fig. 3.3). One week following the assay, 4 animals from this group were injected intraperitoneally with 0.5ml of donor specific alloantibody and 0.5ml of complement. The remaining 4 control animals were injected with a mixture of normal mouse serum and complement. One week after the passive antibody treatment, the iodine uptake was measured again and all 8 animals were found to carry functional grafts (Fig. 3.3). Cytotoxic antibody was also demonstrated in those animals which had been injected with donor specific alloantibody and complement (Table 3.3). Histological examination of the allografts removed the day following the last iodine uptake assay, similarly showed them to be normal (Fig. 3.4).

3.3 DISCUSSION

The results of this study show that neither graft specific antibody alone, nor antibody and complement, have any adverse effect on the function of cultured pancreatic islet and thyroid allografts that are established and revascularized by their host. This finding is in marked contrast to the effect seen when immune serum was administered to animals carrying tolerated but uncultured islets. Frangipane et al. (1977) demonstrated the vulnerability to donor specific antiserum of both AC1 rat islets on WF rats and WF rat islets on immunosuppressed mice. Rats became normoglycaemic within 1 to 6 days and mice within

Figure 3.3

^{125}I uptake by cultured BALB/c thyroid grafted under the renal capsule of allogeneic CBA recipients; before treatment (●), and after treatment with donor specific alloantibody and complement (O), or normal mouse serum and complement (□). The shaded area represents iodine uptake levels up to 2 S.D.'s above the mean uptake by rejected uncultured allografts (BALB/c→CBA) tested 23 days after transplantation. Points outside the shaded area indicate functional transplants.

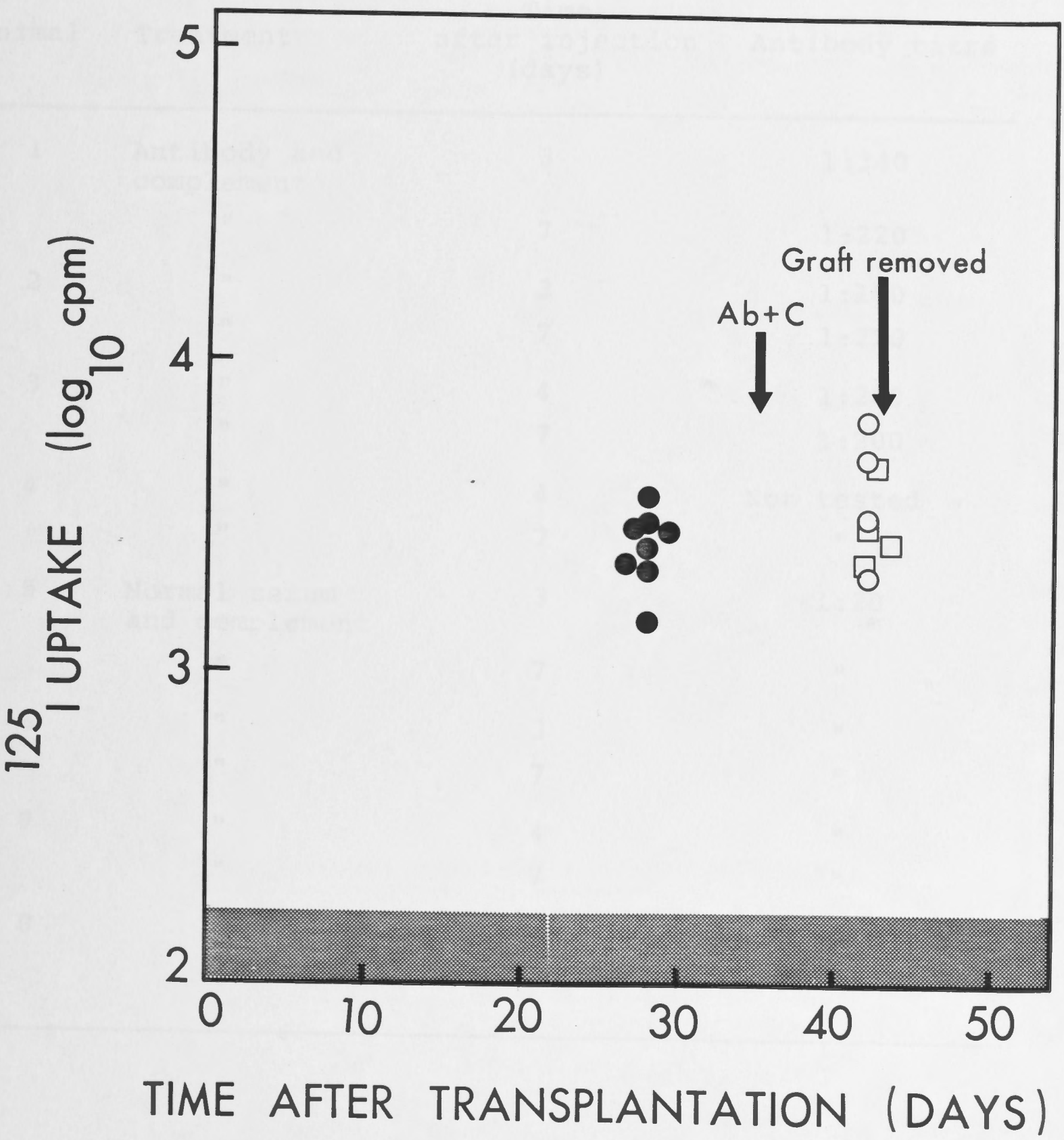

5
4
3
2
125 I UPTAKE (log10 cpm)
Ab+C
Graft removed
0
10
20
30
40
50
TIME AFTER TRANSPLANTATION (DAYS)

Table 3.3

Cytotoxic antibody titres in thyroid allograft recipients

Animal	Treatment	Time after injection (days)	Antibody titre
1	Antibody and complement	3	1:340
	"	7	1:220
2	"	3	1:180
	"	7	1:220
3	"	4	1:260
	"	7	1:200
4	"	4	Not tested
	"	7	"
5	Normal serum and complement	3	<1:20
	"	7	"
6	"	3	"
	"	7	"
7	"	4	"
	"	7	"
8	"	4	"
	"	7	"

Figure 3.4

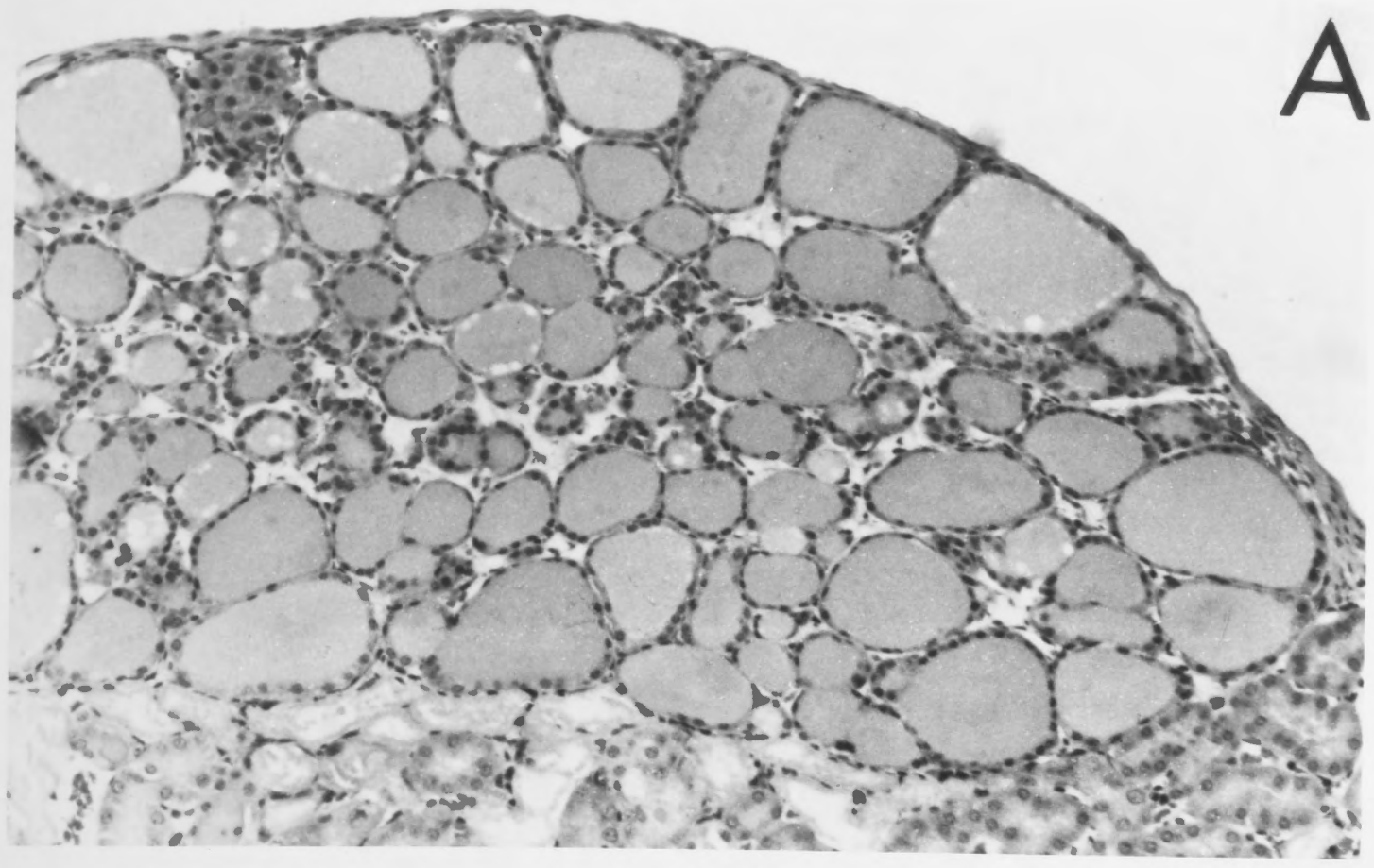
Histological appearance of thyroid allografts.

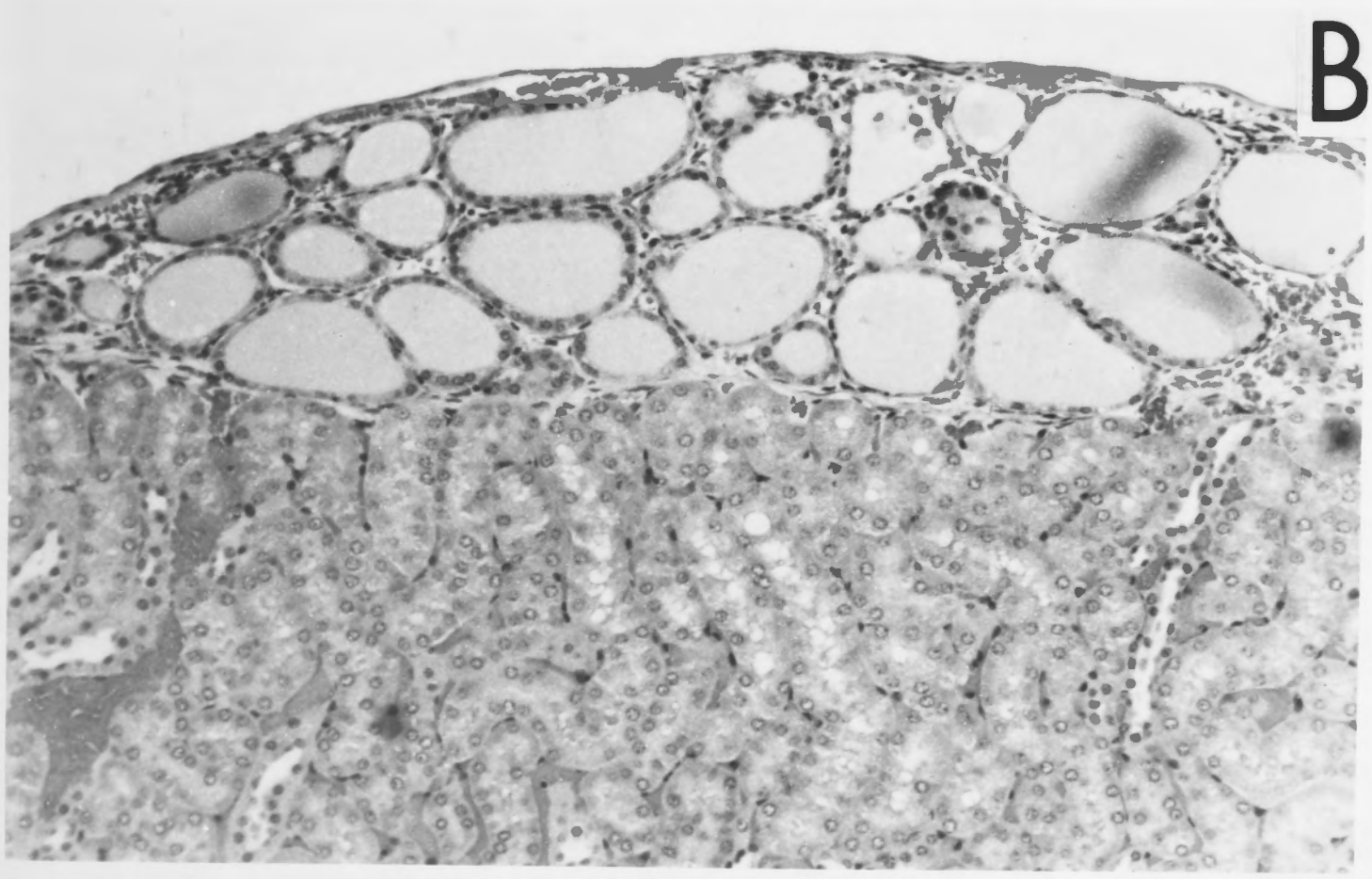
A. Thyroid graft from antibody and complement treated animal.

B. Thyroid graft from control animal.

Note in both the presence of intact thyroid follicles and lack of rejection response (haematoxylin and eosin; x 164).

(Note that these sections are representative of those taken from all experimental animals)


A


B

24 hours after the administration of donor specific anti-serum. Similarly, Naji et al. (1975, 1979a) showed that ACI rat islets and skin transplanted to WF diabetic rats made tolerant to ACI antigens at birth by the intravenous injection of (WFxACI) F_1 bone marrow cells, were vulnerable to WF anti-ACI serum given 21 to 65 days after restoration of normoglycaemia. Hyperglycaemia resulted in 1 to 6 days, although skin grafts were rejected much more slowly. Our finding is also in marked contrast to the effect seen when antibody and complement were administered to animals carrying tolerated kidney or skin allografts (French, 1972; McKenzie and Henning, 1978; Hart et al. 1980; Section 1.5.1.2), that possess vascular endothelium of donor origin. In these studies, grafts were hyperacutely rejected.

Endothelium replacement has been suggested as an explanation for the changes in sensitivity to antibody and complement seen in skin grafts over a period of weeks following transplantation. An initial delay in sensitivity lasting about one week, is rapidly followed by increasing sensitivity, peaking at about 2 weeks and finally moving into a period of resistance by about 5 to 6 weeks (Jooste et al. 1973; Gerlag et al. 1975; Jooste and Winn, 1975; Gerlag et al. 1980; Jooste et al. 1981a). The explanation has been that once the initial vascular network has been established between the graft and the host, graft vascular endothelium is rapidly replaced by host cells, thereby rendering the established skin grafts resistant. In contrast, primary vascularized grafts, where extensive endothelial replacement would not

readily occur, retain their sensitivity for longer periods. Burdick et al. (1979) showed that 56 day established rat to mouse heart xenografts could be rejected by the passive administration of antibody and complement but not similarly established skin of the same donor type and on the same host. Convincing evidence for this proposal of endothelium replacement has come from recent studies of Jooste and her colleagues (Jooste et al. 1981a, b). Rat skin grafts transplanted to mice were removed at various times after transplantation and tested in vitro for reactivity with mouse anti-rat and rat anti-mouse serum. Using immunofluorescence techniques they showed that up to 14 days after grafting, rat or donor cell surface antigens were present but as the loss of sensitivity developed beyond this period, mouse or host cell surface antigens were detected. By day 109, all the graft vascular endothelium was of host origin. Thus, resistance of long-term skin grafts was attributed to the replacement of donor vascular endothelium by host cells. With primary vascularized grafts, on the other hand, it would be expected that donor-type endothelium would persist for more prolonged periods. Hart et al. (1980) have in fact shown that, in contrast to skin, long term (>100 days) surviving DA kidneys on LEW rats are rejected in hyperacute fashion when given donor specific antiserum plus guinea pig complement. In a follow up study (Hart and Fabre, 1981a) using mouse monoclonal antibodies to rat Ia and SD type antigens, they found that at 50 days post transplantation such allografts still possessed donor type endothelium.

In allografts which have been cultured *in vitro*, a different situation exists. Here, the vascular endothelium is already depleted by the time the cultured tissue is ready for transplantation (Parr *et al*. 1980a). In our study, therefore, it would appear that revascularization of the cultured tissue by host endothelium protects the islet and thyroid transplants from an antibody mediated attack.

Using immunoferritin labelling, Parr (1979) reported that in contrast to mouse pancreatic duct, acinar and endothelial cells, beta cells lacked demonstrable H-2 antigen. If this were the case, the revascularized graft would be protected from antibody-mediated attack simply because it lacked recognizable antigen. However, this does not appear to be so and other workers have since shown that pancreatic islet cells do express detectable levels of H-2 antigens. Faustman *et al*. (1980), using direct microcytotoxicity testing, demonstrated the presence of H-2K and H-2D but not Ia antigens from islets in 3 different mouse strains and Parr *et al*. (1980b) confirmed the lack of Ia antigen expression on the endocrine beta cells. Moreover, studies in our own laboratory and elsewhere indicate that both the established islet and thyroid allografts express recognizable antigen because such grafts can be rejected when the recipient is challenged with donor strain leucocytes (Lafferty and Woolnough, 1977; Lacy *et al*. 1979c; Bowen *et al*. 1981). This rejection process is accompanied by a heavy mononuclear infiltrate into the allografts (Lafferty and Woolnough, 1977).

The above discussion suggests that the cellular target of antibody mediated rejection is the vascular endothelium. The target antigens, therefore, are likely to be any expressed on its surface. HLA-A, B, C (class I) antigens which are present on most nucleated cells and HLA-DR (class II) antigens, as well as blood group antigens are likely targets and are expressed on endothelial cells in man (Hirshberg et al. 1979; Hayry et al. 1980; Natali et al. 1981; Scott et al. 1981). Both RT1.A (class I) and RT1.B (class II) antigens have been demonstrated on renal endothelium of ACI rats, although the class II antigens have a more restricted distribution (Paul et al. 1981). Mouse skin can be rejected in hyperacute fashion by antibody directed against class I antigens (McKenzie and Henning, 1978; de Waal et al. 1980) but not by anti-sera directed against class II alloantigens, even when very high doses of sera absorbed with donor strain red blood cells were used (Jansen et al. 1975a). This was confirmed by McKenzie and Henning (1978) using recombinant mouse strain combinations. It was thought that this probably reflects the lack of, or low level expression of class II alloantigens on mouse endothelial cells.

As to the mechanism of hyperacute rejection, several workers have described the likely sequence of events (Williams et al. 1968; Winn et al. 1973; Hume, 1974; Bogman et al. 1980). Initially, the antibody becomes attached to the endothelium of the graft vasculature where complement is fixed and chemotactic factors are released. This results in the attraction of PMN leucocytes which, in turn, adhere to this complex on the endothelium causing further damage.

Using electron microscopy, bridges can be seen between PMN leucocytes and endothelial cells (Williams et al. 1968). The damaged cells in turn attract more polymorphs thereby exacerbating endothelial damage (Bogman et al. 1980). Destruction of the endothelial cells is followed by platelet adherence and production of thrombi, ultimately resulting in the occlusion of vessels and necrosis of tissue.

It would appear, therefore, that revascularization of a graft with host endothelium does not protect the transplant from cell-mediated damage. However, revascularized islet and thyroid allografts are both resistant to the action of antibody and complement.

3.4 SUMMARY

In this chapter we presented evidence indicating that cultured allografts of both islet and thyroid tissue are not susceptible to donor specific antibody and complement. This resistance is thought to be due to a revascularization of the graft tissue by host endothelial cells.

In the following Chapter we will assess the cellular requirements for rejection of cultured islet allografts.

CHAPTER 4

SENSITISED LYT 1^+2^+ CELLS TRIGGER ACUTE REJECTION OF PANCREATIC ISLET ALLOGRAFTS

4.1 INTRODUCTION

For many years graft rejection has been considered a cell mediated function (Snell, 1957; Section 1.5.2), but only recently has direct evidence been obtained that T cells trigger the process of allograft rejection. For example, Hall et al. (1978a) demonstrated that Ig-negative, long-lived, T lymphocytes were responsible for initiating heart allograft rejection in rats; the transfer of cells from sensitised animals showed that memory was carried by long-lived, non-recirculating Ig-negative small lymphocytes (Hall et al. 1978b). More recently, Loveland and co-workers (Loveland et al. 1981; Loveland and McKenzie, 1982a,b; McKenzie, 1983) demonstrated that rejection of skin grafts in adult thymectomized, irradiated, bone marrow reconstituted (ATXBM) mice was triggered by Lyt 1^+2^- T cells from sensitised animals and drew analogies between graft rejection and delayed type hyper-sensitivity (DTH) reactions. Although direct evidence was lacking, they concluded that Lyt 1^+2^+ cells were not involved in skin graft rejection. This conclusion was also based on the assumption that the Lyt 1^+2^- and Lyt 1^+2^+ T cell subsets have quite distinct functions. In a similar study, Dallman and Mason (1982) also found that skin allograft rejection in ATXBM rats could be restored by the injection of T helper ($0X8^-$) cells.

The functions of lymphocyte subpopulations have been the subject of controversy for some time, with claims that Lyt 1^+2^- or "helper" T cells have properties quite distinct from the Lyt 1^+2^+ subclass (Bach et al. 1976; Larsson et al. 1980; Wagner et al. 1980, reviewed by Swain and

Dutton, 1980). Lymphokine production, help for B cell responses and DTH reactions were considered to be the role of the Lyt 1^+2^- subset. However, considerable evidence has accummulated which suggests that it is incorrect to make a rigid distinction between the properties of the Lyt 1^+2^- and Lyt 1^+2^+ subsets. Cells of the Lyt 1^+2^- subset can also mediate a cytotoxic effector function (Dennert and Yamagata, 1980). Similarly, the Lyt 1^+2^+ subset, the traditional cytotoxic effector subset, can also be cytotoxic and release lymphokine (Andrus et al. 1981), provide help for B cells (Swain and Panfili, 1979) and mediate DTH (Leung and Ada, 1980).

In view of these findings, we examined the cellular requirements for rejection of cultured pancreatic islet allografts which, unlike the skin grafts, can survive indefinitely in fully immunocompetent recipients (Bowen et al. 1980). The results demonstrate that the transfer of specifically sensitised cells, (but not of normal or cells sensitised to unrelated antigens) to animals carrying cultured islet allografts, triggers the acute rejection process. In vitro sensitised cells were also ineffective in triggering rejection. Treatment of the sensitised cells with either anti-Thy 1.2 antibody and complement or anti-Lyt 2.1 antibody and complement abolished the ability of the cells to trigger rejection.

4.2 RESULTS

4.2.1. Transfer of in vivo sensitised cells triggers rejection of islet allografts

Four CBA mice which had been carrying functional islet allografts ranging from 42-383 days, were injected intra-

venously with 5×10^7 spleen cells from CBA mice previously immunized with P815. The total cytotoxic activity injected was 5.8 to 6.0 $\log_{10}$ cytotoxic units (Table 4.1). The blood glucose concentrations and the body weight of these animals were closely monitored. The islet allografts were acutely rejected following the cell transfer, with blood glucose concentrations rising out of the normal range in all animals within 6 days of cell transfer. A concomitant drop in body weight was observed (Fig. 4.1). In experiments where blood glucose readings were taken daily, animals challenged in this way showed consistent rejection at 3 days after cell transfer (Fig. 4.2; Chapter 5). It is also evident from these data (Fig. 4.1) that grafts are susceptible to attack irrespective of the length of time they have been in the recipient. Grafts which had been in the recipient for almost 400 days were just as susceptible to rejection as those which had been in the animal for 40-50 days.

Grafts were removed from the mice 1-2 weeks after challenge with sensitised cells and evaluated histologically. Tissue sections were examined to determine the degree, if any, of islet damage and mononuclear cell infiltration in and around the transplant tissue. The grafts from these animals contained very little or no islet tissue, with patches of scar tissue and a light to heavy mononuclear cell infiltration evident (Fig. 4.3).

Table 4.1.

Cytotoxic activity (log_{10} cytotoxic units) of spleen cells transferred

Spleen cells transferred	Mouse No.	Total cytotoxic activity
Normal CBA	1	N.T.
	2	N.T.
CBA anti-P815	3	5.8
	4	6.0
	5	5.8
	6	5.8
CBA anti-EL4	7	5.6
	8	5.6
CBA anti-P815 treated with complement	9	5.8
	10	5.5
	11	5.0
	12	5.0
	13	5.4
anti-Thy 1.2 + complement	14	<4.2
	15	<4.0
	16	<3.8
	17	<3.8
anti-Lyt 2.1 + complement	18	<3.8
	19	<3.8
	20	<4.0
	21	<4.0

All mice received 5×10^7 spleen cells or remaining cells after treatment with antibody and complement. The cytotoxic activity is that measured on the immunising cell.
N.T. not tested.

Figure 4.1

Non-fasting blood glucose levels (□) and body weight (●) of four CBA mice carrying functional islet allografts and injected intravenously with 5×10^7 CBA anti-P815 spleen cells, between 42 and 383 days post transplantation (shown by arrows). The shaded region indicates the range of normal blood glucose concentrations. All allografts were acutely rejected irrespective of the time of cell transfer after transplantation.

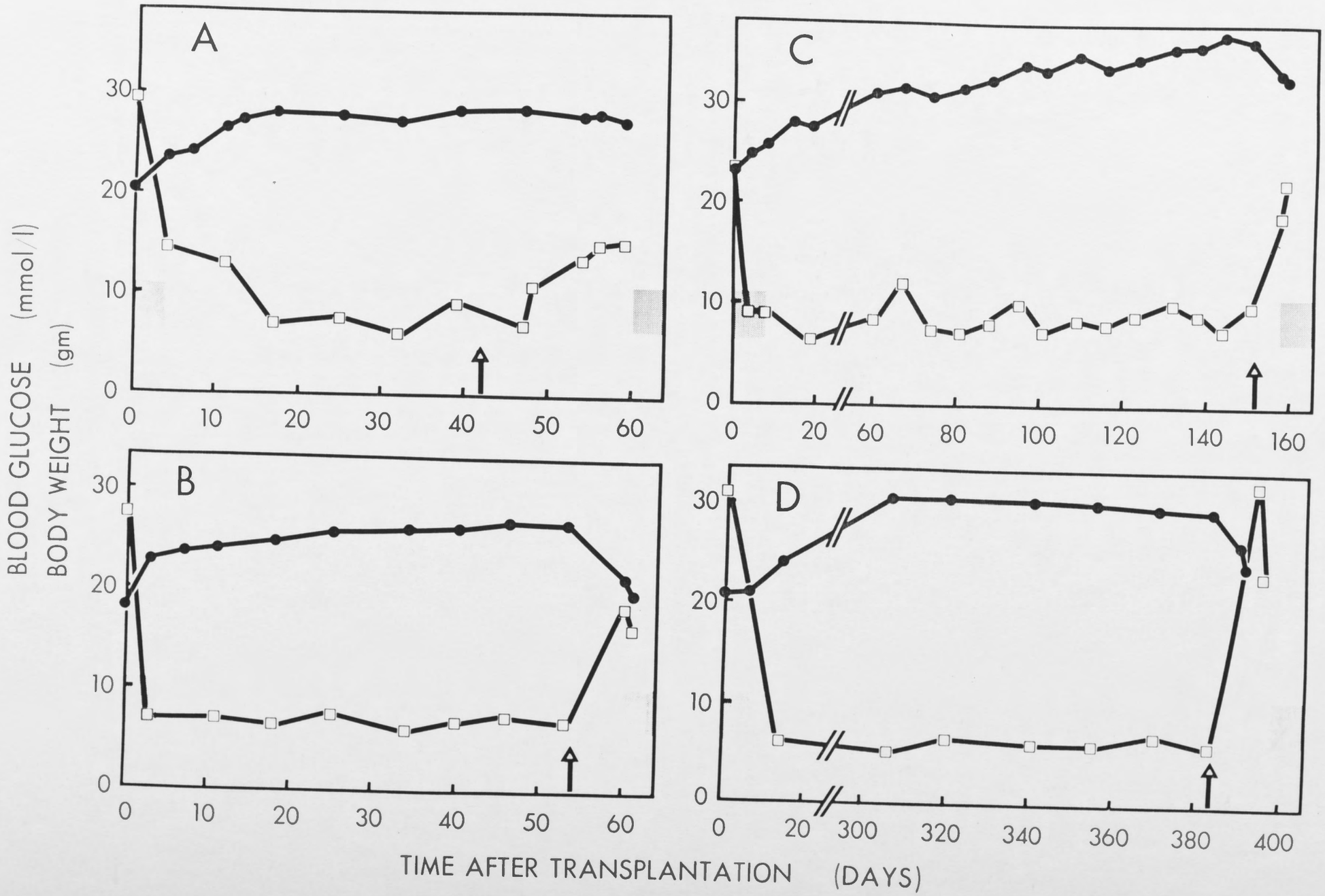
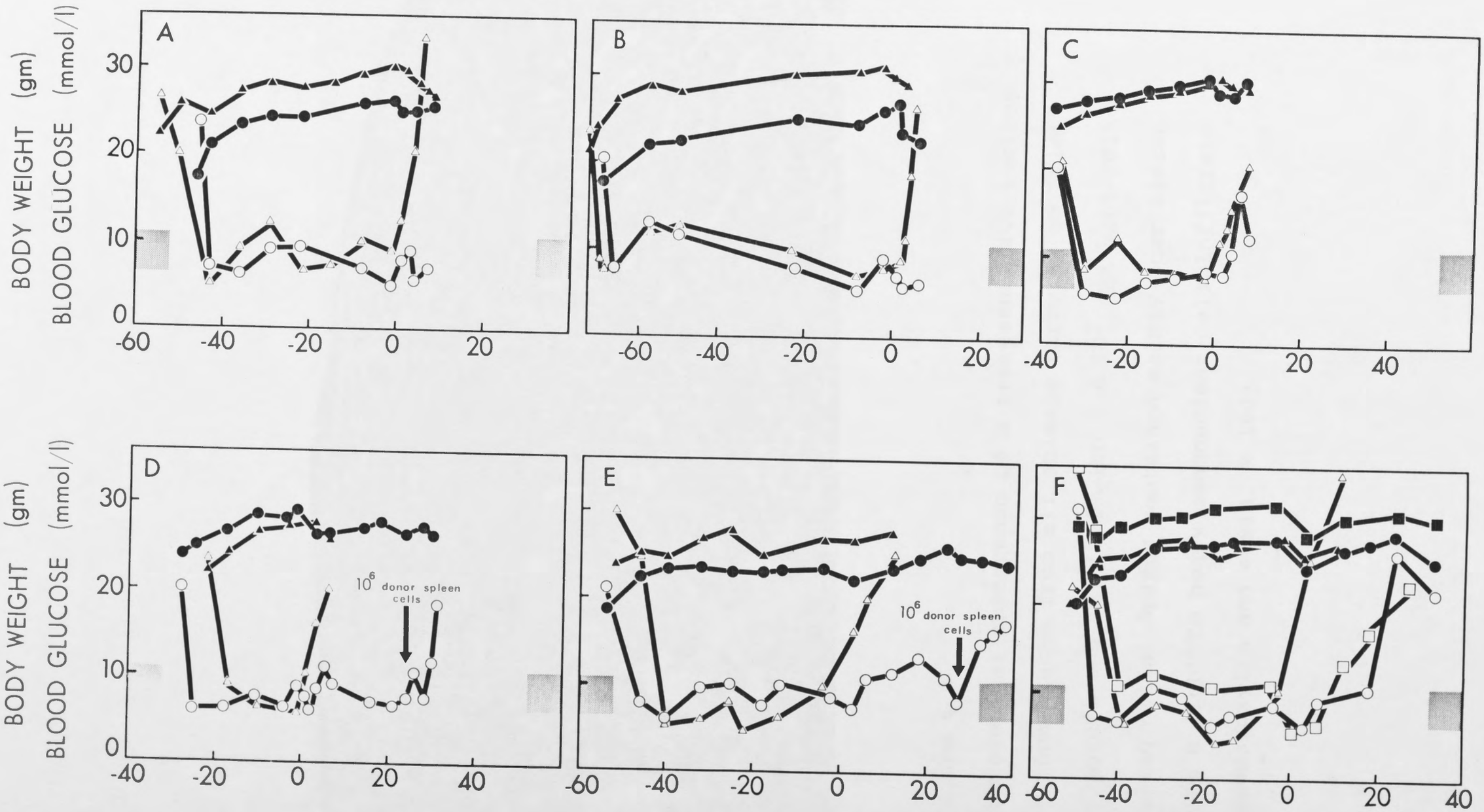

A
B
C
D
BLOOD GLUCOSE (mmol/l)
BODY WEIGHT (gm)
TIME AFTER TRANSPLANTATION (DAYS)

Figure 4.2

Non-fasting glucose levels of 7 Cyclosporine treated animals (O,□) and 6 control animals (Δ), and their body weight respectively (●,), (▲). All mice were injected with 5 x 10^7 CBA anti-P815 spleen cells at day 0. Transplantation of animals occurred between 30-70 days before injection of sensitised cells, with a reversal of diabetes occurring within 1-2 weeks of transplantation. Note the rapid rejection (by 3 days following injection of sensitised cells) in control animals.


BODY WEIGHT (gm)
BLOOD GLUCOSE (mmol/l)
A
B
C
D
E
F
30
20
10
0
-60
-40
-20
0
20
40
10^6 donor spleen cells
TIME FROM INJECTION OF SENSITISED CELLS (DAYS)

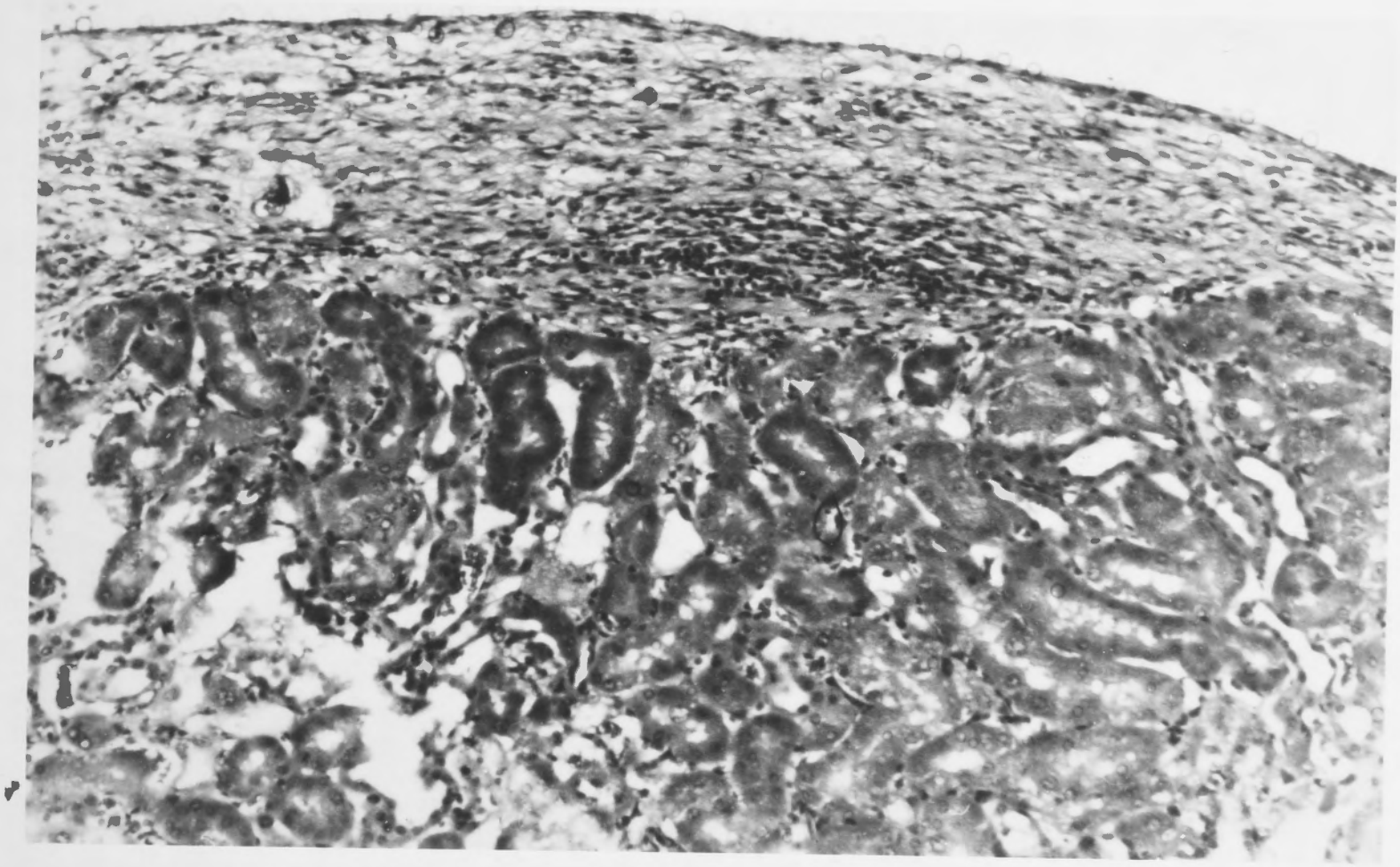


Figure 4.3

Histological appearance of a representative section of those taken from all animals carrying an islet allograft and injected with 5×10^7 CBA anti-P815 spleen cells. Grafts contained mainly scar tissue with a light to heavy mononuclear cell infiltrate (Haematoxylin and eosin, x 164).

4.2.2. Failure of passively transferred, in vitro sensitised CBA anti-H-2^d cells to reject islet allografts

Since spleen cells from immunized mice could trigger the rejection of established allografts, it was of great interest to determine if cells sensitised *in vitro* could perform the same function. Five CBA mice carrying BALB/c islet allografts were injected intravenously with 1 to 4.5 x 10^7 CBA lymph node cells which had been sensitised against BALB/c spleen cells *in vitro*. These transferred cells failed to trigger graft rejection irrespective of the time of transfer (Table 4.2) and despite their high cytotoxic potential when compared with *in vivo* activated cells (Table 4.1).

4.2.3. Failure of normal cells or anti-H-2^b cells to trigger rejection of established islet allografts

Since sensitised cells could trigger the rejection of established allografts, it was important to determine if normal cells or cells sensitised against a third party H-2 haplotype, H-2^b, could trigger rejection.

Two CBA mice carrying functional BALB/c islet allografts were injected intravenously with 5 x 10^7 normal CBA spleen cells. These animals remained normoglycaemic (Fig. 4.4) indicating that increased numbers of normal cells do not lead to allograft rejection. About 3 weeks later these animals were injected with 5 x 10^7 spleen cells from CBA mice which had been immunized with the H-2^b tumour, EL-4. The total cytotoxic activity of the injected cells measured on H-2^b targets was 5.6 $\log_{10}$ cytotoxic units (Table 4.1) and there was no detectable cytotoxic activity on H-2^d targets. The mice still

Table 4.2.

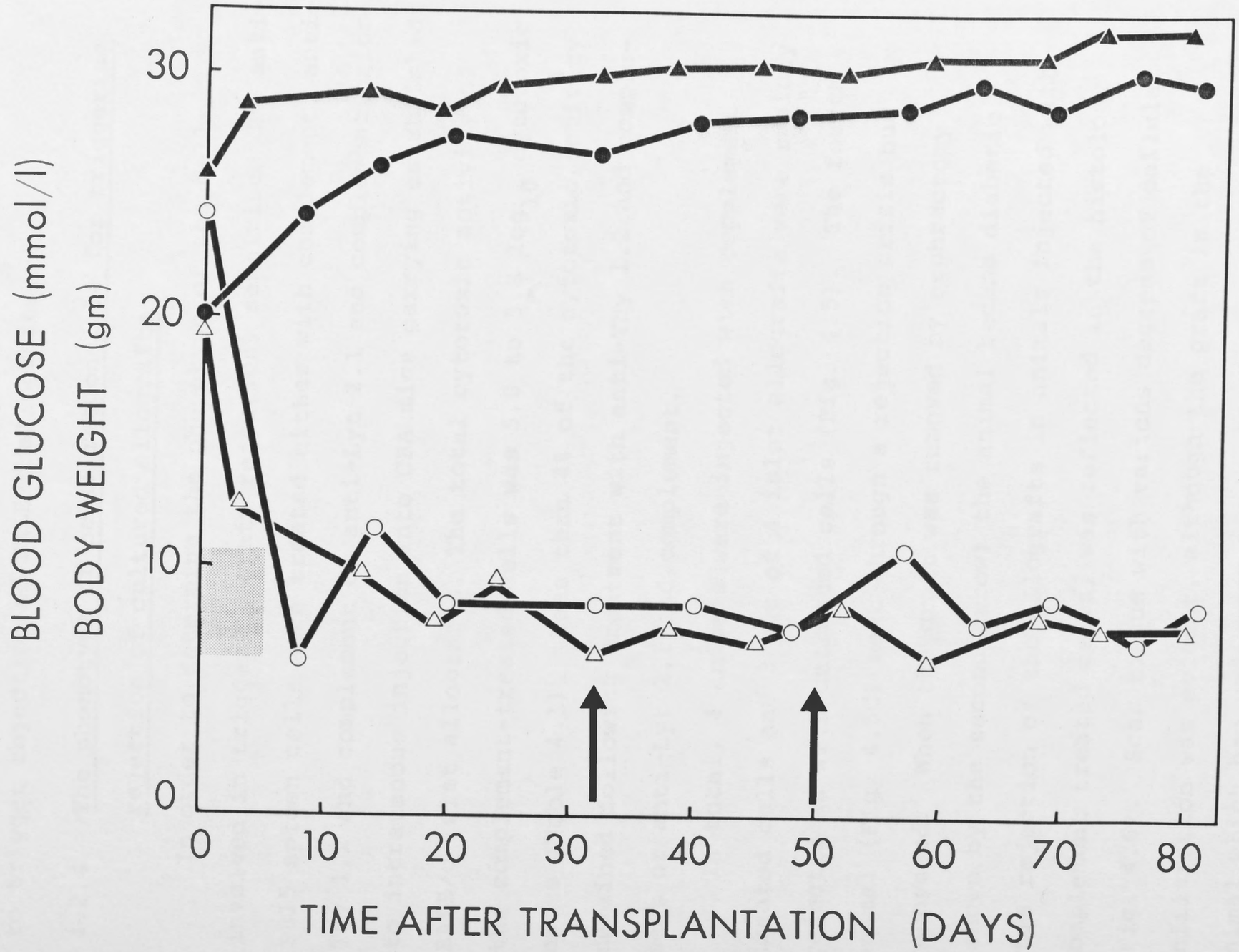
Effect on BALB/ c islet allografts of passive transfer of CBA anti-H-2^d cells sensitised *in vitro*

Animal No.	No. days post transplant	No. cells injected ($x10^7$)	Cytotoxic activity log_{10} C.U/10^6 cells	Total cytotoxic activity	Effect*
1	256	1.0	5.5	6.5	None
2	246	1.0	5.5	6.5	None
3	48	2.0	5.5	6.8	None
4	50	1.5	5.8	7.0	None
5	47	4.5	5.2	6.9	None

* Effect on blood glucose

Figure 4.4

Non-fasting blood glucose levels (Δ, O) and body weight (▲,●) of 2 CBA mice carrying functional islet allografts and injected intravenously with normal CBA spleen cells and 5×10^7 CBA anti-H-2^b spleen cells at 32 and 50 days post-transplantation respectively (shown by arrows). Both animals remained normoglycaemic.


BLOOD GLUCOSE (mmol/l)
BODY WEIGHT (gm)
30
20
10
0
0
10
20
30
40
50
60
70
80
TIME AFTER TRANSPLANTATION (DAYS)

remained normoglycaemic (Fig. 4.4), indicating that cells with cytotoxic activity against H-2^b alloantigens are unable to trigger rejection of BALB/c allografts.

4.2.4. The phenotype of cells responsible for triggering rejection of cultured allografts

In order to determine the phenotype of the cells involved in triggering graft rejection, sensitised CBA anti-P815 spleen cells were treated either with complement, anti-Thy 1.2 and complement or anti-Lyt 2.1 and complement prior to intravenous injection into CBA mice carrying established BALB/c islet allografts. The total cytotoxic activity of the complement-treated cells was 5.0 to 5.8 $\log_{10}$ cytotoxic units (Table 4.1). Less than 4% of the cytotoxic activity remained following treatment with anti-Thy 1.2 and complement or anti-Lyt 2.1 and complement.

In total, 4 animals were injected with complement treated cells and 3 out of 4 islet allografts were rapidly rejected as with untreated cells (Fig. 4.5). The fourth animal (Fig. 4.5C) went through a rejection crisis but recovered. When the graft was removed by nephrectomy (shown by the second arrow) the animal became diabetic. This rejection of the allografts in animals injected with complement treated cells, was reflected in the histology (Fig. 4.6). Scar tissue with various degrees of cellular infiltration was evident, although the graft in the animal which had undergone a rejection crisis still contained remnants of islet tissue surrounded by pockets of heavy cellular infiltration (Fig. 4.6B,C).

Four animals were injected with anti-Thy 1.2 and complement treated cells. This abolished the ability of

Figure 4.5

Non-fasting blood glucose levels (□) and body weight (●) of 4 CBA mice transplanted with BALB/c islets and injected with 5×10^7 complement treated CBA anti-P815 spleen cells at the times indicated by the arrows. Three out of 4 animals rapidly rejected their graft; the fourth (panel C) went through a rejection crisis but recovered. Removal of its graft by nephrectomy (indicated by the second arrow) led to its hyperglycaemia.

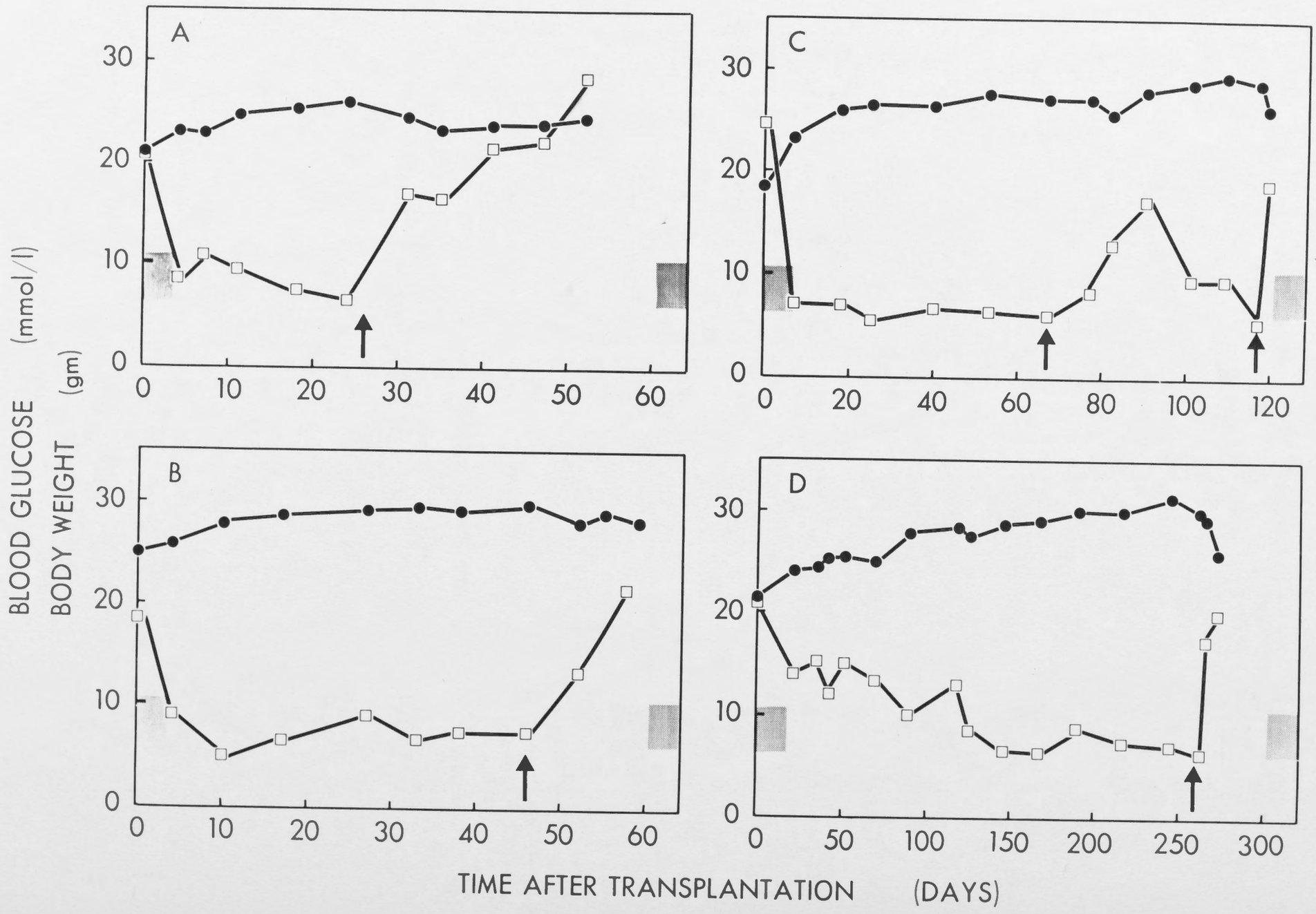

A
B
C
D
30
20
10
0
0
10
20
30
40
50
60
0
20
40
60
80
100
120
0
50
100
150
200
250
300
BLOOD GLUCOSE (mmol/l)
BODY WEIGHT (gm)
TIME AFTER TRANSPLANTATION (DAYS)

Figure 4.6

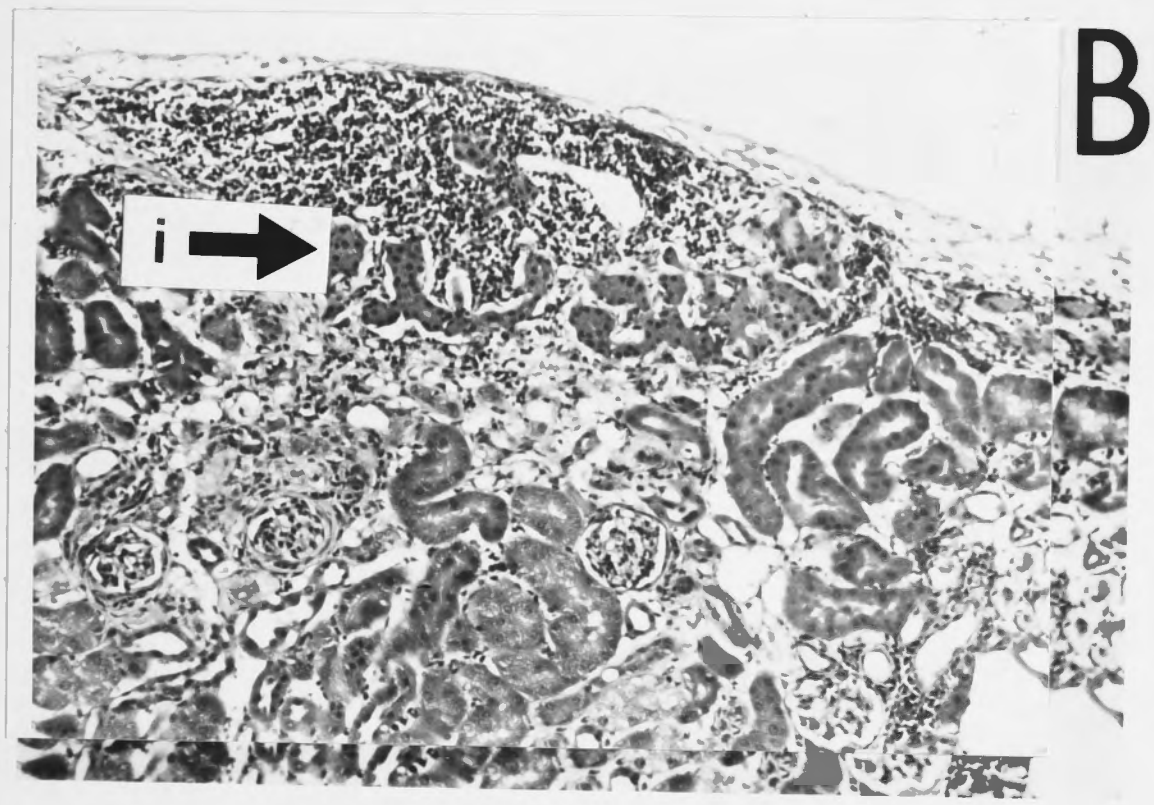
Histological appearance of islet allografts from 2 representative animals which had been injected with 5×10^7 CBA anti-P815 spleen cells treated with complement.

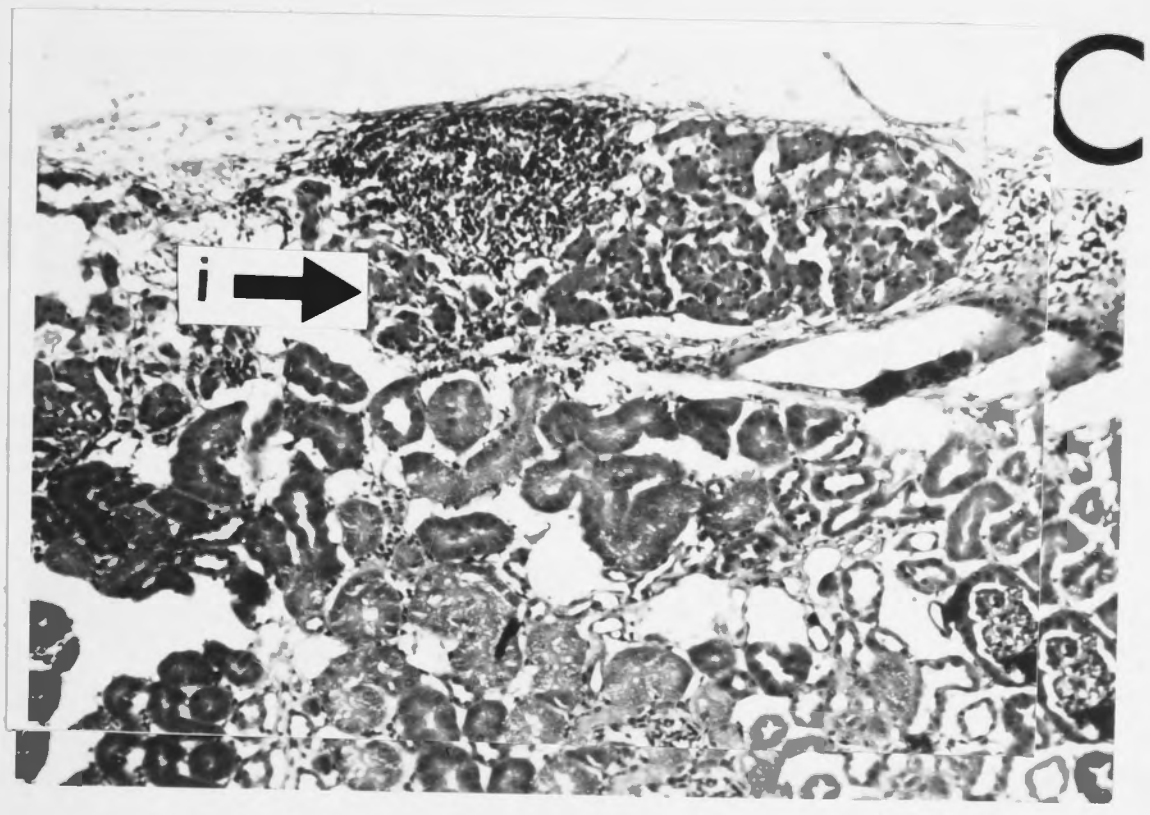
A. Section stained with haematoxylin and eosin showing scar tissue (x109).

B. Section of graft from animal which had undergone a rejection crisis (haematoxylin and eosin; x109). Note the remnants of islet tissue (i) surrounded by pockets of heavy cellular infiltration.

C. Section from the same animal as B, stained with aldehyde fuchsin (x109) showing the dark staining granulated beta cells (i) present in the remnant islet tissue.


A


B
i


C
i

sensitised cells to trigger the rejection process (Fig. 4.7). Histologically the grafts showed intact islet tissue with the presence of a few pockets of cellular infiltration in some animals (Fig. 4.8). A further 4 animals were injected with anti-Lyt 2.1 and complement treated cells and this treatment similarly abolished the ability to reject the islet allografts (Fig. 4.9). This was confirmed histologically by the presence of intact islet tissue (Fig. 4.10).

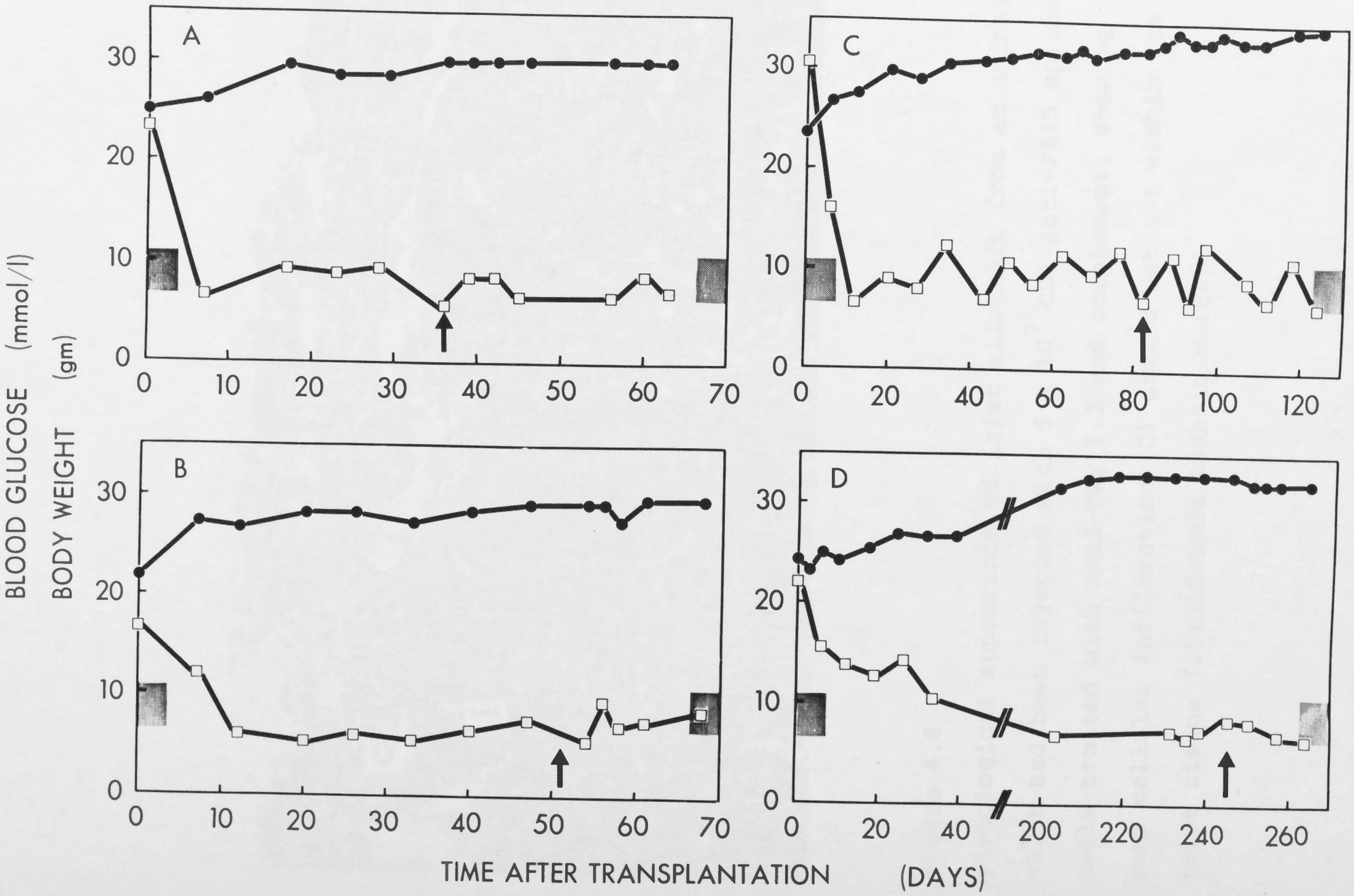
Thus, sensitised Lyt 1^+2^+ cells are responsible for triggering an acute rejection of cultured pancreatic islet allografts.

4.3 DISCUSSION

The results with cultured islet allografts show that *in vivo* sensitised Lyt 1^+2^+ cells trigger an acute rejection of the allograft. In the experimental model used in this study, culture of islet tissue in 95% oxygen allows allografts to survive indefinitely in immunocompetent allogeneic recipients (Bowen *et al.* 1980; reviewed by Prowse *et al.* 1982a). This provides a model which can be used to unequivocally analyse the cells involved in triggering rejection because, unlike most other cell transfer systems, grafts are not rejected in untreated animals, nor in animals which receive normal cells. The complication of grafts rejecting spontaneously in immunoincompetent or immuno-impaired animals, or following the transfer of normal or nonspecifically activated cells (Rouse and Wagner, 1972; Corley and Kindred, 1977; Rosenstein *et al.* 1981; Loveland and McKenzie, 1982b) makes the interpretation of results difficult. Similar rejection times seen

Figure 4.7

Non-fasting glucose levels (□) and body weight (●) of 4 CBA mice transplanted with BALB/c islets and injected with 5×10^7 anti-thy 1.2 and complement treated CBA anti-P815 spleen cells at the times indicated by the arrows. All animals remained normoglycaemic.


A
B
C
D
30
20
10
0
0
10
20
30
40
50
60
70
80
100
120
200
220
240
260
BLOOD GLUCOSE (mmol/l)
BODY WEIGHT (gm)
TIME AFTER TRANSPLANTATION (DAYS)

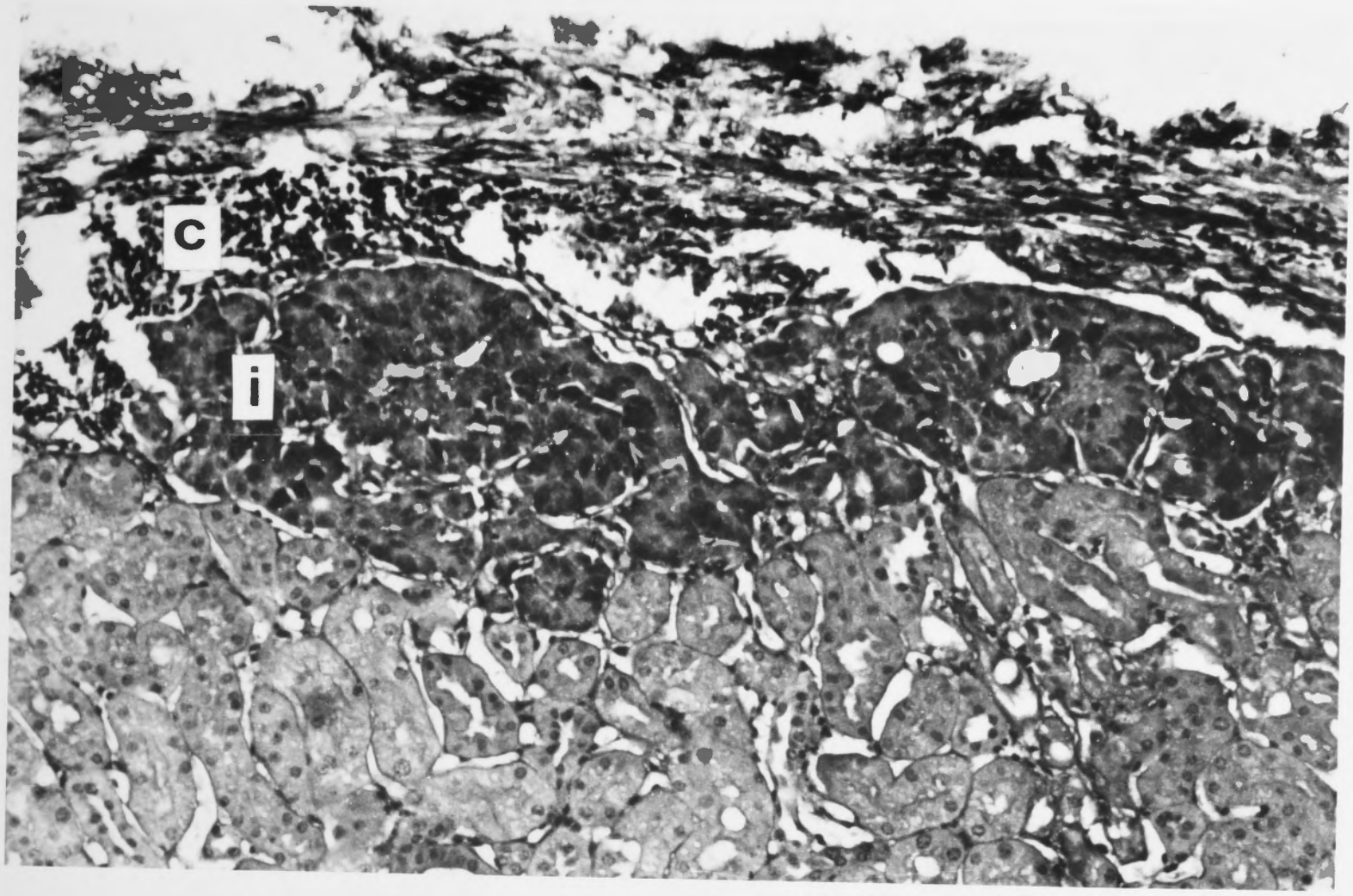
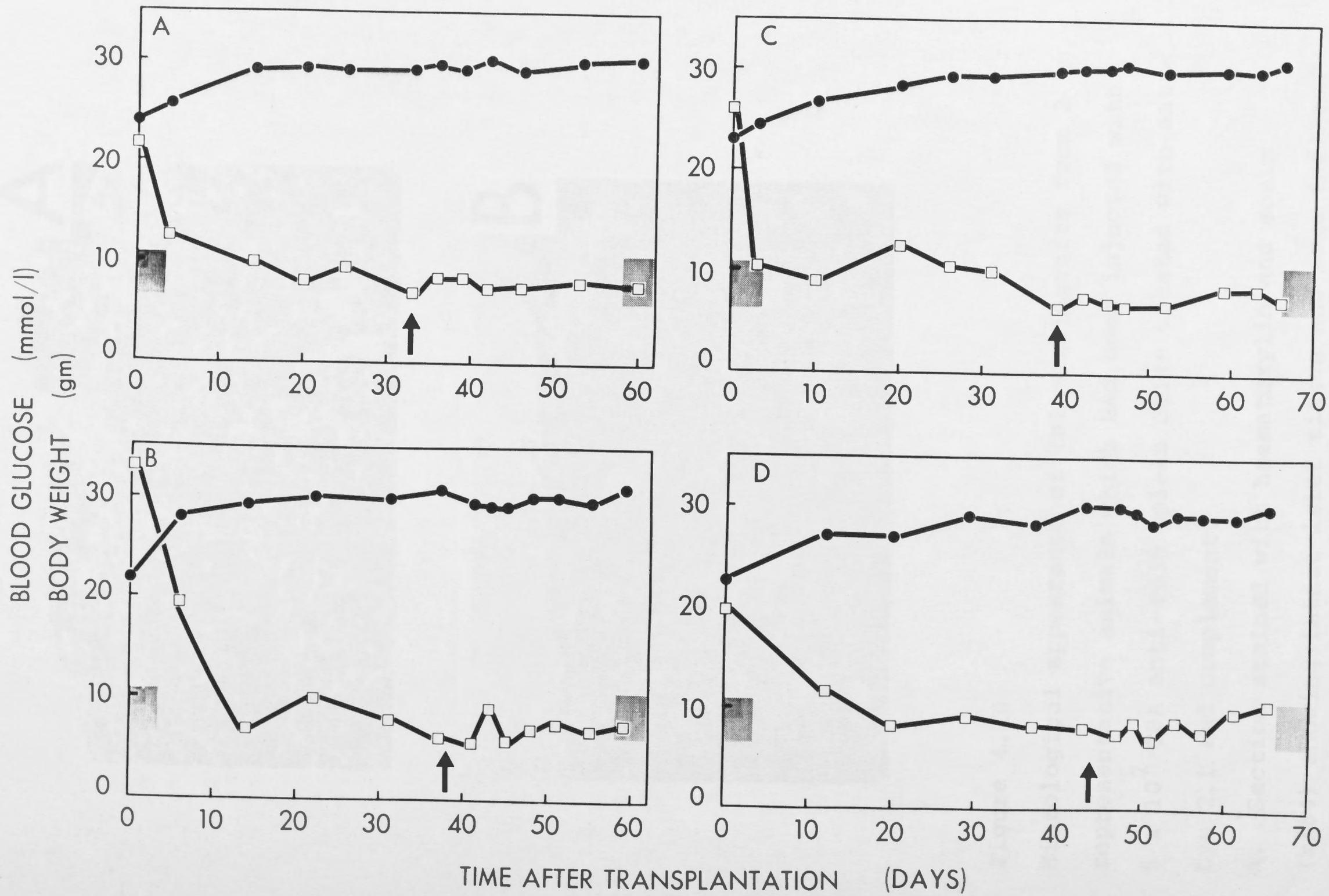


Figure 4.8

Histological appearance of islet allograft from an animal which had been injected with 5×10^7 CBA anti-P815 spleen cells treated with anti-Thy 1.2 and complement, showing some cellular infiltration (C) around but not within the islet tissue (i) (aldehyde fuchsin x 164).

Figure 4.9

Non-fasting blood glucose levels (□) and body weight (●) of 4 CBA mice transplanted with BALB/c islets and injected with 5×10^7 anti-Lyt 2.1 and complement treated CBA anti-P815 spleen cells at the times indicated by the arrows. All animals remained normoglycaemic.


A
B
C
D
30
20
10
0
0
10
20
30
40
50
60
70
BLOOD GLUCOSE (mmol/l)
BODY WEIGHT (gm)
TIME AFTER TRANSPLANTATION (DAYS)

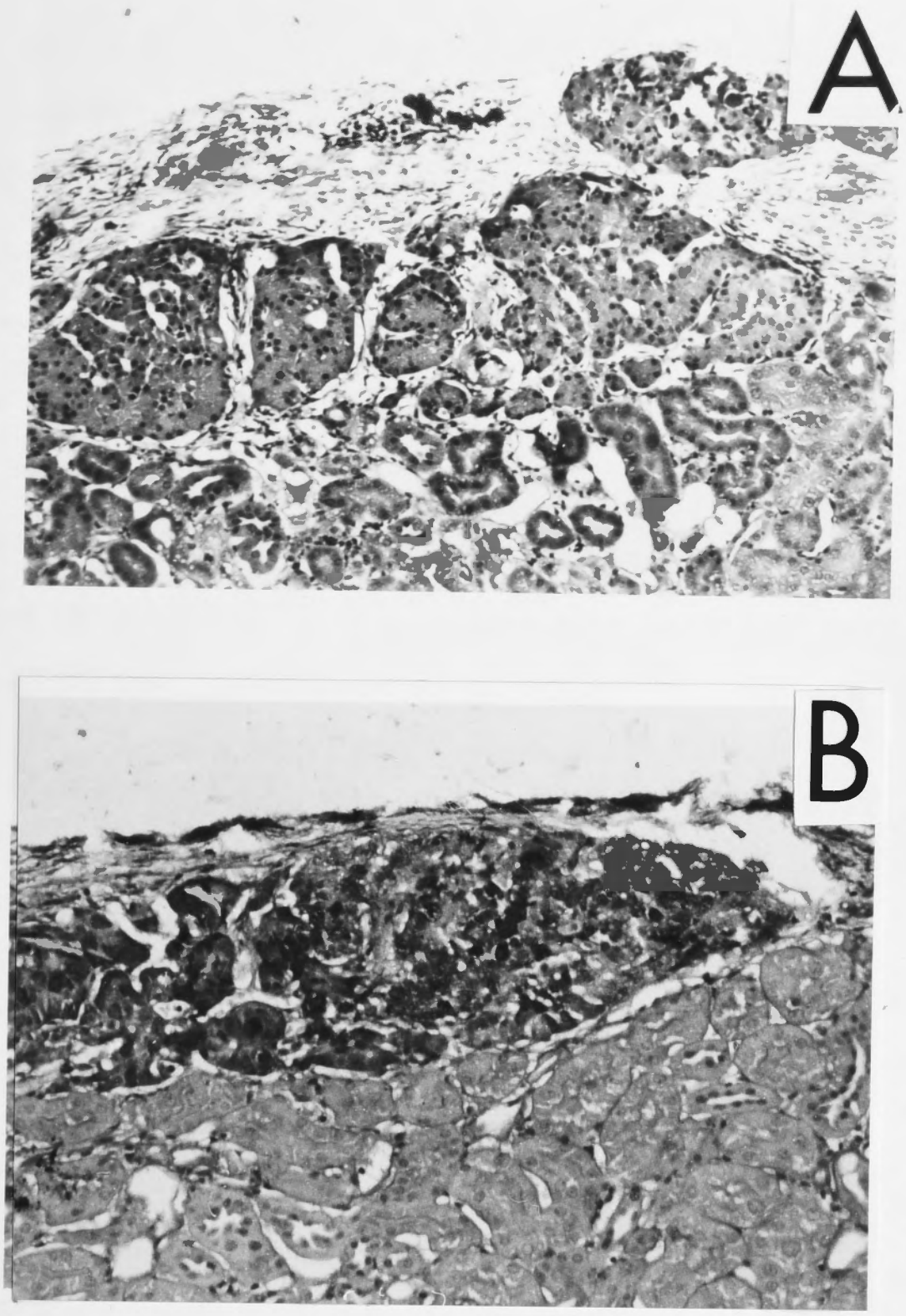


Figure 4.10

Histological appearance of islet allografts from 2 representative animals which had been injected with 5×10^7 CBA anti-P815 spleen cells treated with anti-Lyt 2.1 and complement.

A. Section stained with haematoxylin and eosin (x164) showing intact islet tissue and some infiltration.

B. Section stained with aldehyde fuchsin (x109) showing the dark staining beta cells of the islet tissue.

following transfer of normal cells or cells activated against a third party H-2 haplotype and specifically sensitised cells (Corley and Kindred, 1977; Loveland et al. 1981; Loveland and McKenzie, 1982b) suggests that differentiation and/or sensitisation could be occurring before rejection takes place.

Our results with spleen cells from animals sensitised against a third party H-2 haplotype ($H\text{-}2^b$), show that these cells were unable to trigger rejection, showing that the rejection process is a specific phenomenon. It is worth noting that in agreement with Rouse and Wagner (1972), cytotoxic cells activated against $H\text{-}2^b$ alloantigens showed negligible crossreactive cytotoxic activity on $H\text{-}2^d$ targets.

Another approach to the analysis of cellular rejection has been to study the effect of *in vitro* activated cytotoxic cells on graft survival - a method which has long been promoted as a good correlate of allograft rejection (Wagner *et al*. 1973). In our study, donor lymph node cells which were sensitised *in vitro* had no effect on allograft survival. This was somewhat surprising in view of the findings of other workers who demonstrated *in vivo* function of *in vitro* sensitised cells (Rouse and Wagner, 1972; Corley and Kindred, 1977; Rosenstein *et al*. 1981). However, although these workers reported that *in vitro* sensitised cells were capable of accelerating allogeneic skin graft rejection, the effect did not correlate with the cytotoxic activity of the cell preparations (Rosenstein *et al*. 1981) and in one model, cells failed to reject grafts in lethally irradiated mice (Rouse and

Wagner, 1972). It is possible that the *in vivo* effectiveness of *in vitro* activated cells is compromised by the poor recirculating ability of these cells. Lotze *et al*. (1980) found that *in vitro* activated and labelled cells injected into mice and humans, were largely removed from the circulation by early trapping in the lungs and subsequently in the liver and spleen. In an allograft situation, such removal would obviously limit their contact with graft tissue. In tumour allograft models, attempts to show *in vivo* function of *in vitro* generated effector cells have generally met with limited success and Engers et al. (1982) have shown that in their model system the route of injection also plays a role.

As already indicated, the role of Lyt 1^+2^- lymphocytes in graft rejection has received prominence through studies with ATXBM mice by Loveland and coworkers (Loveland *et al*. 1981; Loveland and McKenzie, 1982a,b), who challenged the concept that cytotoxic T cells (Lyt 1^+2^+) mediate rejection. They proposed that it is the Lyt 1^+2^- T cell which is responsible for rejection and, in contrast, the Lyt 1+2+ cell plays no role, although a positive selection of these cells to test their ability to trigger rejection was not attempted. More recently they further characterised the Thy 1^+, Lyt 1^+2^- cell causing skin graft rejection in the mouse, as having the Lyt $1^+2^-5^+6^-7^+$ phenotype; cytotoxic T cells carry the Lyt $5^+6^+7^-$ phenotype (Loveland and McKenzie, 1982c).

Studies by Vesole *et al*. (1982) on the cellular requirements of cultured thyroid allografts, also seemed to indicate a role for Lyt 1^+2^- cells, although the cell type mediating rejection was not determined. They showed

that, not only was there frequently a long delay in rejection following adoptive immunization, but also a lack of correlation between the presence of cytotoxic cells in the innoculum and the capacity to trigger graft rejection. In a more recent study, however, Warren et al. (unpublished data), have shown that cultured thyroid allografts do require sensitised Lyt 1^+2^+ cells to trigger rejection. Thus, while Lyt 1^+2^- T cells may mediate skin rejection, the available data suggest it would be incorrect to conclude that cytotoxic or Lyt 1^+2^+ T cells play no role in graft rejection.

Our data conclusively show that Thy 1^+, Lyt 1^+2^+ lymphocytes can trigger islet allograft rejection and while this conflicts with the findings of Loveland et al. (1981), the two findings can be reconciled. In considering interactions between sensitised cells and grafts, it is important to recognise that differences exist in cellular content and antigen expression between skin, organ grafts and cultured tissue. The above studies suggest that the subclass of T lymphocytes primarily responsible for triggering allograft rejection, probably depends on the type of tissue grafted, which in turn reflects the particular class of alloantigen expressed. Cultured islet and thyroid allografts express only class I antigens (Parr et al. 1980b) whereas cells present in the skin grafts express both class I and class II antigens (Hammerling, 1976). Since the traditional view of T lymphocyte activation is that class I and class II antigen cause the activation of Lyt 1^+2^+ and Lyt 1^+2^- T cells respectively (Cantor and Boyse, 1976; Swain and Panfili, 1979; Ledbetter et al. 1980), then it would be expected that there could be differences in cellular requirements

for rejection of skin and islet grafts. The cell donors used by Loveland et al. (1981) were immunized by skin grafts and cells from these animals would include Lyt 1^+2^- cells activated against class II alloantigens and Lyt 1^+2^+ cells activated against class I alloantigens. The elimination of Lyt 1^+2^+ cells could leave sensitised Lyt 1^+2^- cells capable of recognising and responding to class II antigens on responsive blood cells in skin grafts. Such an interaction would result in an inflammatory reaction and destruction of graft tissue (Lafferty and Talmage, 1976). In contrast, the sensitised cells used in our study were from animals immunized with P815 which expresses class I but not class II alloantigens. This would lead to the activation of Lyt 1^+2^+ cells and, as already indicated, the only target antigens on the parenchymal cells of cultured islet and thyroid allografts are of class I (Parr et al. 1980b). Therefore, elimination of Lyt 1^+2^+ cells would eliminate cells capable of recognizing alloantigens on the graft. It should, however, be noted that there are some anomalies to the above explanation. Lyt 1^+2^- cells from sensitised animals were found to be active in rejecting skin grafts differing only at the H-2K (class I) locus (Loveland and McKenzie, 1982b) and sensitised Lyt 1^+2^- T cells also induced the rejection of allogeneic EL-4 lymphoma which does not carry class II alloantigens (Loveland and McKenzie, 1982a).

We have shown, therefore, that in the case of cultured islet tissue, Lyt 1^+2^+ cells are important in allograft rejection - a rejection which may be mediated through lymphokine release (Chapter 5). Taking our data together

with the findings of Loveland and coworkers, both Lyt 1^+2^- and Lyt 1^+2^+ cells can trigger graft rejection; the actual effector cell phenotype being determined by the antigens on the immunising tissue and the antigens expressed on the graft.

4.4 SUMMARY

In this Chapter we presented data to show that cultured islet allografts in immunocompetent animals are very rapidly rejected following the passive transfer of in vivo sensitised cells. Non-specific or normal cells did not trigger rejection. Similarly, it was shown that in vitro sensitised cells are not effective in triggering rejection.

The cells triggering rejection were found to belong to the Thy 1^+, Lyt 1^+2^+ and not the Lyt 1^+2^- subpopulation of T lymphocytes. The apparent conflict between our findings and that of other workers was discussed.

In the following chapter (Chapter 5) we extend our investigations into the mode of function of Lyt 1^+2^+ cells in rejecting islet allografts.

CHAPTER 5

CYCLOSPORINE: A TOOL FOR ANALYSING MODE OF FUNCTION OF LYT 1^+2^+ CELLS IN ISLET ALLOGRAFT REJECTION

5.1 INTRODUCTION

Cyclosporine (CyA) has, since the demonstration of its immunosuppressive activity and purification in the mid 1970's (Borel, 1980), made an important impact as a drug in clinical immunosuppression (for recent reviews see Green, 1982; White and Calne, 1982; Beveridge, 1983; Thomson, 1983). This has come about because of several important advantages it has over other immunosuppressive drugs, including its very low myelotoxicity and lymphocyte specificity which leaves undamaged the animal's phagocytic cell function (Borel, 1981; Britton and Palacios, 1982). While it is not without its side effects, particularly that of nephrotoxicity, its superior features has enabled its use to generally give better clinical results, especially in bone marrow and renal transplantation, than those obtained with conventional immunosuppressive therapy (Beveridge, 1983).

CyA has also made an impact as an important immunological tool. Yet, while there is agreement that CyA affects a variety of T cell functions (see Britton and Palacios, 1982; Thomson, 1983), the precise mode of action of CyA is still under discussion. Two basic models (reviewed by Lafferty et al. 1983b) have been proposed to account for the activity of CyA. The subset model, which claims that the different T cell subsets are functionally different from one another and react differently to CyA, postulates that the drug exerts its effect by inhibition of the helper subset (Bunjes et al. (1981). The signalling model, which sees all T cells as having the same potential, proposes that CyA interferes

with the transmission of the antigen specific signal within the T cell following antigen binding (Andrus et al. 1981). Irrespective of the conflicting interpretation of CyA action, however, its differential effects on activated T cell function have been widely confirmed. Lyt 1^+2^+ cells are both cytotoxic and can also release lymphokine (Andrus et al. 1981). CyA has the differential effect on activated T cells of preventing lymphokine release without affecting the cell's cytotoxic function (Borel, 1981; Bunjes et al. 1981; Andrus and Lafferty, 1982; Orosz et al. 1982). This drug can, therefore, be used as an analytical tool to discriminate between these two functions.

The acute nature of the rejection of cultured islet allografts by Lyt 1^+2^+ cells demonstrated in Chapter 4, suggested that this rejection was due to cells of the cytotoxic subset. In this Chapter we have used CyA to investigate which function of activated Lyt 1^+2^+ cells is responsible for rejection of islet allografts. The results indicate that lymphokine release plays an important role.

5.2 RESULTS

Data presented in Chapter 4 has shown that cultured islet allografts are rejected by Thy 1^+, Lyt 1^+2^+ T cells. In this chapter we tested for the mode of action of Lyt 1^+2^+ cells by treating recipients of pancreatic islet allografts with subcutaneous injections of 75 mg/kg of CyA daily, beginning at day $^-1$ to day 7 following the intravenous injection of 5×10^7 CBA anti-P815 spleen cells. The total cytotoxic activity measured in vitro of the

injected cells, was 5.4 to 6.1 $\log_{10}$ cytotoxic units.

A total of 7 CBA mice carrying established islet allografts were treated with CyA and injected with sensitised CBA spleen cells, generally between day 30 to 50 post transplantation. Another group of 6 animals, also carrying islet allografts but not treated with CyA, were used as controls. These were similarly injected with 5×10^7 sensitised spleen cells. Blood glucose concentrations and body weight were determined daily for the first week and thereafter as required. Six of the CyA treated animals failed to reject their graft following the transfer of sensitised cells (Fig. 5.1). The seventh animal (Fig. 5.1 Panel C) appeared to have undergone a rejection crisis and was on its way to recovery. At this point it was killed to examine its graft histologically. Unfortunately, the presence of numerous adhesions resulted in the loss of the graft tissue. In contrast, the untreated control animals consistently rejected their graft within 3 days of the cell transfer (Fig. 5.1). Histologically, the grafts from animals treated with CyA showed intact islet tissue with some pockets of cellular infiltration around the graft (Fig. 5.2 A, B). Grafts from untreated control animals showed massive cellular infiltration with essentially no islet tissue remaining intact (Fig. 5.2C). These results indicate that lymphokine release by Lyt 1^+2^+ cells is of major importance in the rejection of cultured islet allografts.

It was also of interest to determine whether animals which had been treated with CyA, developed tolerance once the drug treatment ceased. In 4 CyA treated animals, the blood glucose levels and body weight were monitored for

Figure 5.1

Non-fasting blood glucose levels of 7 CyA treated animals (O,□) and 6 control animals (Δ), and their body weight respectively (●,■), (▲). All mice were injected with 5×10^7 CBA anti-P815 spleen cells at day 0. CyA injections were given from day $^-1$ to day 7 following injection of sensitised cells. Transplantation of animals occurred between 30-70 days before injection of sensitised cells, with a reversal of diabetes occurring within 1-2 weeks.

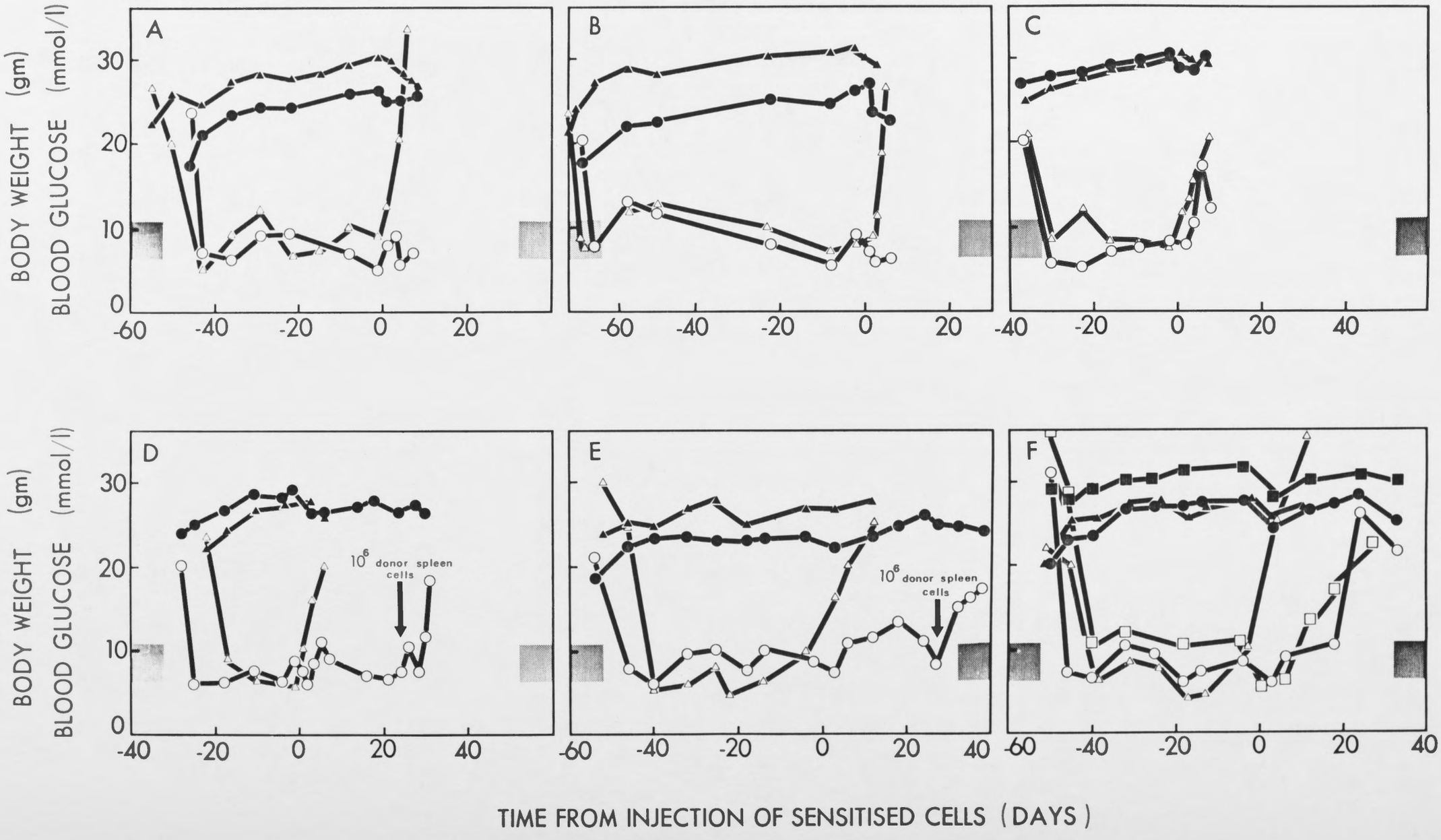
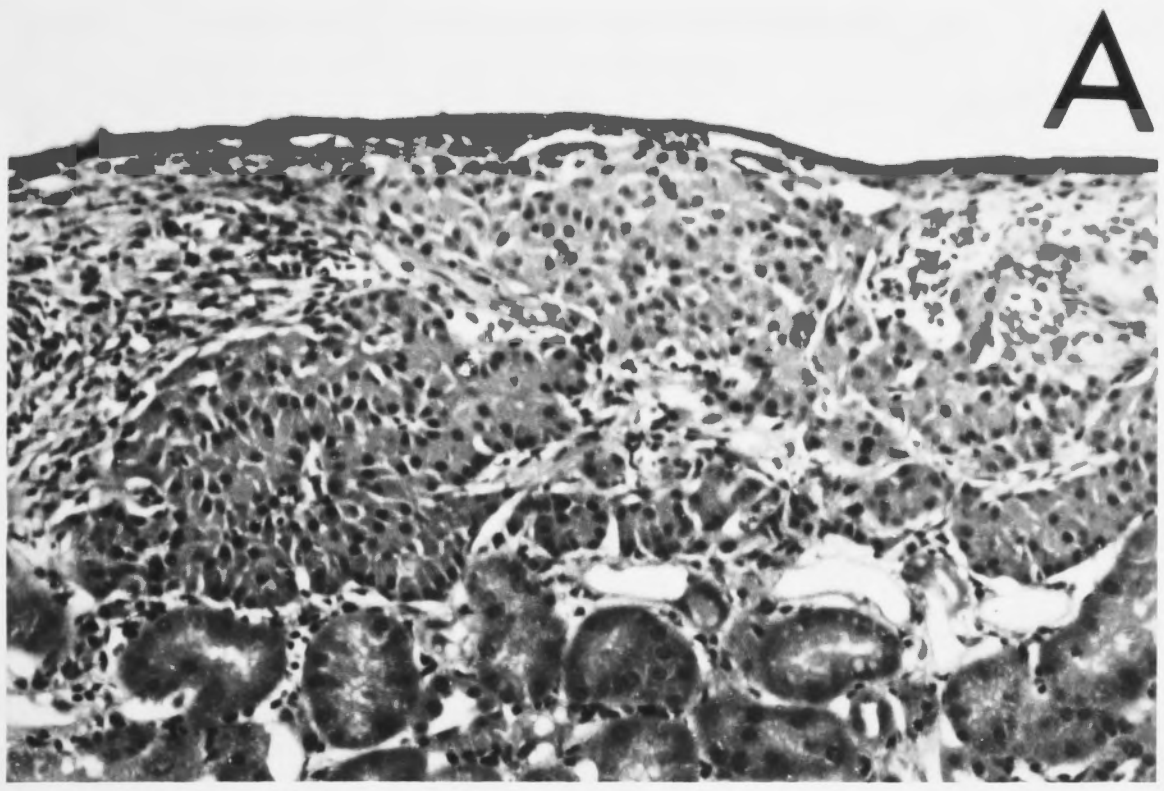
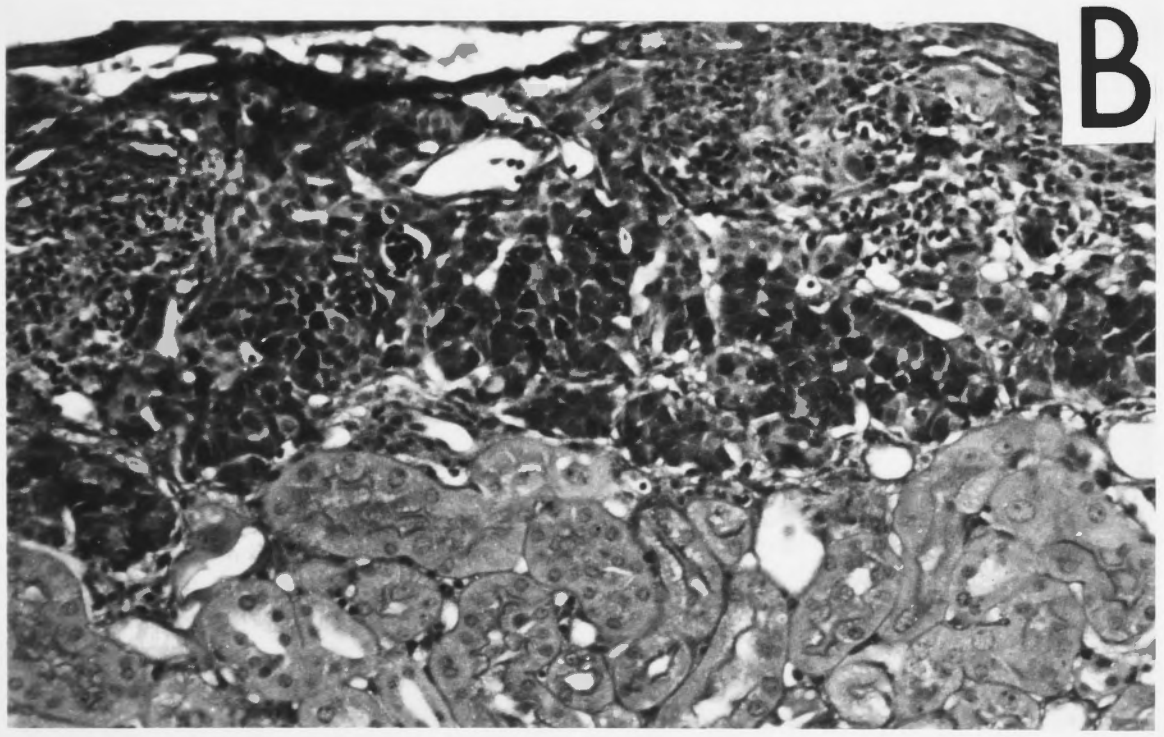

BODY WEIGHT (gm)
BLOOD GLUCOSE (mmol/l)
A
B
C
D
E
F
30
20
10
0
-60
-40
-20
0
20
40
10^6 donor spleen cells
TIME FROM INJECTION OF SENSITISED CELLS (DAYS)

Figure 5.2

Histological appearance of representative islet allografts following challenge with sensitised spleen cells. A, graft from CyA treated animal (haematoxylin and eosin, x 109); B, graft from a similarly treated animal showing the dark staining insulin producing beta cells (aldehyde fuchsin, x 109); C, graft from an untreated control showing complete infiltration (haematoxylin and eosin, x 109).


A


B


C

several weeks following the withdrawal of the drug treatment. Two animals immediately rejected their graft (Fig. 5.1, Panel F). One animal appeared to go through a minor rejection crisis but then returned to within the normal blood glucose range (Fig. 5.1, Panel E). This and another CyA treated animal (Fig. 5.1 Panel D) were injected intraperitoneally 3 weeks later with 10^6 normal spleen cells of donor origin. In both animals, this challenge with donor type cells led to the rapid rejection of the graft. The treatment with CyA, therefore, had not induced tolerance to donor antigens.

5.3 DISCUSSION

As demonstrated in Chapter 4, Lyt 1^+2^+ cells are required to trigger the rejection of cultured pancreatic islet allografts. Although rejection may occur through a direct cytotoxic attack on islet cells, or through lymphokine release from Lyt 1^+2^+ cells (Andrus et al. 1981) and an associated inflammatory response, our data showing the acute rejection of islet allografts was thought to be more consistent with a direct cytotoxic effect of the immune cells on transplanted islet cells. The results presented in this Chapter, using CyA as a tool to discriminate between the two functions, shows that the drug prevents rejection by sensitised Lyt 1^+2^+ T cells. This suggests the cytotoxic activity alone is not a sufficient requirement for graft rejection by this cell population. Animals treated with CyA still showed pockets of cellular infiltration around the grafts, although histologically the islet tissue itself appeared intact. These inflammatory foci may be associated with damage to the kidney capsule and

underlying parenchyma tissue produced at the time of transplantation. It is also possible that these pockets of cells represent that proportion of transferred cells which managed to circulate and attach themselves to the graft tissue. Once attached, however, inhibition to release lymphokine abolished their ability to recruit more effector cells to the graft site and hence abolished their ability to reject the islet allograft.

The data presented in this study indicates, therefore, that lymphokine release is important, although the precise role that lymphokine plays remains uncertain. This interpretation does not exclude a role for the cytotoxic function of these cells, as lymphokine may be required to maintain the growth and viability of the activated T cells, or to attract other sensitised cytotoxic cells to the site, thereby magnifying the reaction. Against the first possibility is the fact that two animals rejected their graft when CyA was withdrawn (Fig. 5.1, Panel F), indicating that the T cells were still viable up to that point in time. Some evidence that cytotoxicity by itself can play a role in rejection, is seen in the animals which went through a rejection crisis (Fig. 5.1, Panels C and E). However, this evidence is not sufficient to totally exclude the possibility that cytotoxicity plays no role and that lymphokine release by itself leads to allograft rejection. It would be of interest to deliver the sensitised cells directly into the graft site under the kidney capsule and determine whether the onslaught of such a large number of cytotoxic cells would still prevent the graft from being rejected in a CyA treated animal. An interesting feature

is that recovery can occur when the drug is withdrawn.

The findings from this study are consistent with other work from our laboratory where CyA was used to determine which function is responsible for the local GVH reaction produced when T cells sensitised to class I MHC antigens are injected into the footpad of mice bearing the priming antigen (Hodgkin et al. 1983). Such sensitised cells induce footpad swelling within 24 hours of injection. CyA does not affect the cytotoxic activity of these cells, but does inhibit lymphokine release. Treatment of recipient animals with CyA, using 75mg/kg on day $^{-}$1, 0 and 1, inhibited the expression of the local GVH reaction. In vitro treatment of cells with 1µg/ml of CyA for 1 hour before injection into the footpad, resulted in a delayed response and a reduced peak in footpad swelling compared to controls (Hodgkin - personal communication). That is, the effect of CyA on cells treated in vitro wears off and these cells recover some lymphokine releasing function. However, by treating the cells as well as the recipient, this recovery is prevented and the swelling reaction is completely inhibited.

The question of whether CyA can induce tolerance has been investigated by a number of workers, with some conflicting results, although the majority have reported varying degrees of tolerance following CyA treatment (White et al. 1980; Reece-Smith et al. 1981; Deeg et al. 1982; Du Toit et al. 1982; Nagao et al. 1982; Bordes-Aznar et al. 1983; Hess et al. 1983). With our system, which is quite different to those used by these workers, we were not able to induce tolerance.

However, one interesting finding from these reports has been that following withdrawal of CyA and then challenge with a second donor specific graft, the degree of responsiveness varies with time; that is, graft acceptance passes through different phases so that, with the passage of time, the degree of specificity and stability of tolerance increases (White et al. 1980; Nagao et al. 1982; Bordes-Aznar et al. 1983). Thus, a short course of CyA treatment can lead to indefinite survival even after treatment with the drug has ceased. Until this tolerant state is reached, however, rejection occurs; that is, the graft is in a metastable state. This aspect is explored in the following Chapter. It seems that the drug permits the initial success in grafting and the continued provision of graft antigen is then required for the development of tolerance without the need for the continuous presence of the drug (Kasahara et al. 1982; White, 1983).

5.4. SUMMARY

CyA was used as an analytical tool to determine the mode of function of Lyt 1^+2^+ cells in their rejection of cultured islet allografts. The data show that lymphokine production is required for adequate function of the Lyt 1^+2^+ subset which is responsible for rejection. In this system, treatment with CyA does not lead to the induction of transplantation tolerance. In the following Chapter, we will assess the stabilization of islet allografts and induction of tolerance.

CHAPTER 6

STABILIZATION OF ISLET ALLOGRAFTS BY TREATMENT OF RECIPIENTS WITH ULTRAVIOLET IRRADIATED DONOR SPLEEN CELLS - INDUCTION OF ADULT TOLERANCE

6.1 INTRODUCTION

Removal or inactivation by organ culture, of leucocytes associated with mouse pancreatic islets and thyroid tissue prior to transplantation, allows the successful grafting of these tissues to normal MHC incompatible recipient animals, where the allografts remain functional indefinitely (Lafferty and Woolnough, 1977; Bowen et al. 1980; Donohoe et al. 1983). However, although the immunogenicity of these tissues can be reduced by organ culture, such treatment does not prevent their rejection when the recipient is challenged with lymphoreticular cells of donor origin at, or for some time after transplantation (Lafferty et al. 1976b; Talmage et al. 1976; Lafferty and Woolnough, 1977; Simeonovic et al. 1980; Bowen et al. 1981; Vesole et al. 1982). Rat islet allografts have similarly been rejected following challenge with peritoneal cells syngeneic to the islet donor (Lacy et al. 1979c). Therefore, the immediate post-transplantation period is one of graft vulnerability.

The length of this vulnerable period is limited. An earlier study from our laboratory, aimed at defining the vulnerability of an established pancreatic islet allograft in mice, showed that functional tolerance develops in a proportion of recipients of long-standing allografts ($\geq$100 days) (Bowen et al. 1981). Zitron et al. (1981b) reported a similar finding in the rat. The same phenomenon was also seen in mice grafted with thyroid tissues where, after prolonged residence in the recipient (>100$\leq$350 days), only a proportion of allografts were rejected when the recipient was challenged with 10^5 followed by 10^6 perito-

neal cells of donor origin (Donohoe et al. 1983). Vesole et al. (1982) earlier reported a similar finding in mice carrying cultured thyroid allografts. Only 50% of such animals rejected their graft when challenged with 5×10^6 spleen cells of donor origin; 73% of those rejected had been established in the recipient for less than 6 months. Thus, although there is some variation between different tissues, it does seem that, as the post-transplantation period increases, the allograft becomes progressively less susceptible to rejection. Grafts which cannot be rejected following challenge of the recipient with donor spleen cells, are considered to have moved from the metastable post-transplantation phase into a phase characterized by a stable interaction with the host.

The major question under study here concerns whether the metastable phase can be shortened by appropriate treatment of the recipient. The hypothesis tested is that stabilization can be achieved by the administration to recipients of donor antigen alone; such antigen is provided by ultraviolet (UV) killed spleen cells (Lafferty et al. 1974).

In this study, spleen cells of donor origin were killed with UV light before injection into recipients carrying metastable islet allografts. Following this treatment, recipients were tolerant to a challenge of live spleen cells. Graft stability was therefore hastened by treatment with UV irradiated cells. Moreover, this graft stabilization is shown to result from the induction of specific tolerance to tissues of donor origin.

6.2 RESULTS

6.2.1. Immunogenicity of UV irradiated spleen cells

The capacity of killed (UV irradiated) and live spleen cells to induce allograft rejection was compared. Fourteen diabetic CBA mice were each transplanted with 7 cultured BALB/c islet clusters (350 islets). Following the return of the blood glucose level to normal, nine of these diabetes-reversed animals were injected (intraperitoneally) with 10^6 UV irradiated spleen cells of donor origin at about 30 days post-transplantation. The remaining five animals were challenged with an intraperitoneal injection of 10^6 live spleen cells of donor origin at day 30.

The results of this study show that, in general, UV irradiated cells do not induce allograft rejection (Fig. 6.1). Of the nine animals injected with UV irradiated cells, only one rejected its graft (Fig 6.1H). Even after a further two doses of UV irradiated cells, the blood glucose level in the remaining eight animals (Fig. 6.1 A-G, I), remained within the normal range and body weight continued to increase. In contrast, of the five animals challenged with 10^6 live spleen cells at day 30, all rejected their graft (Fig. 6.2). All five animals returned to the diabetic condition by 6-15 days after challenge. UV irradiated spleen cells, therefore, are very much less immunogenic than living spleen cells.

6.2.2. UV irradiated cells stabilize the islet allograft

A total of 9 animals received 3 doses at weekly intervals, of 10^6 UV irradiated cells. Eight failed to reject their graft following this treatment and were then challenged (intraperitoneally) two weeks after the final dose of UV irradia-

Figure 6.1

Non-fasting blood glucose levels (□) and body weight (■) of 9 CBA mice transplanted with 350 cultured allogeneic (BALB/c) islets and treated with UV-irradiated spleen cells of donor origin from about 30 days after transplantation. Only 1 animal (H) rejected its graft after this treatment. Each of the other 8 animals was then challenged with 10^6 followed by 10^7 live cells of donor origin. Only one animal (I) rejected its graft after injection with 10^6 live cells.

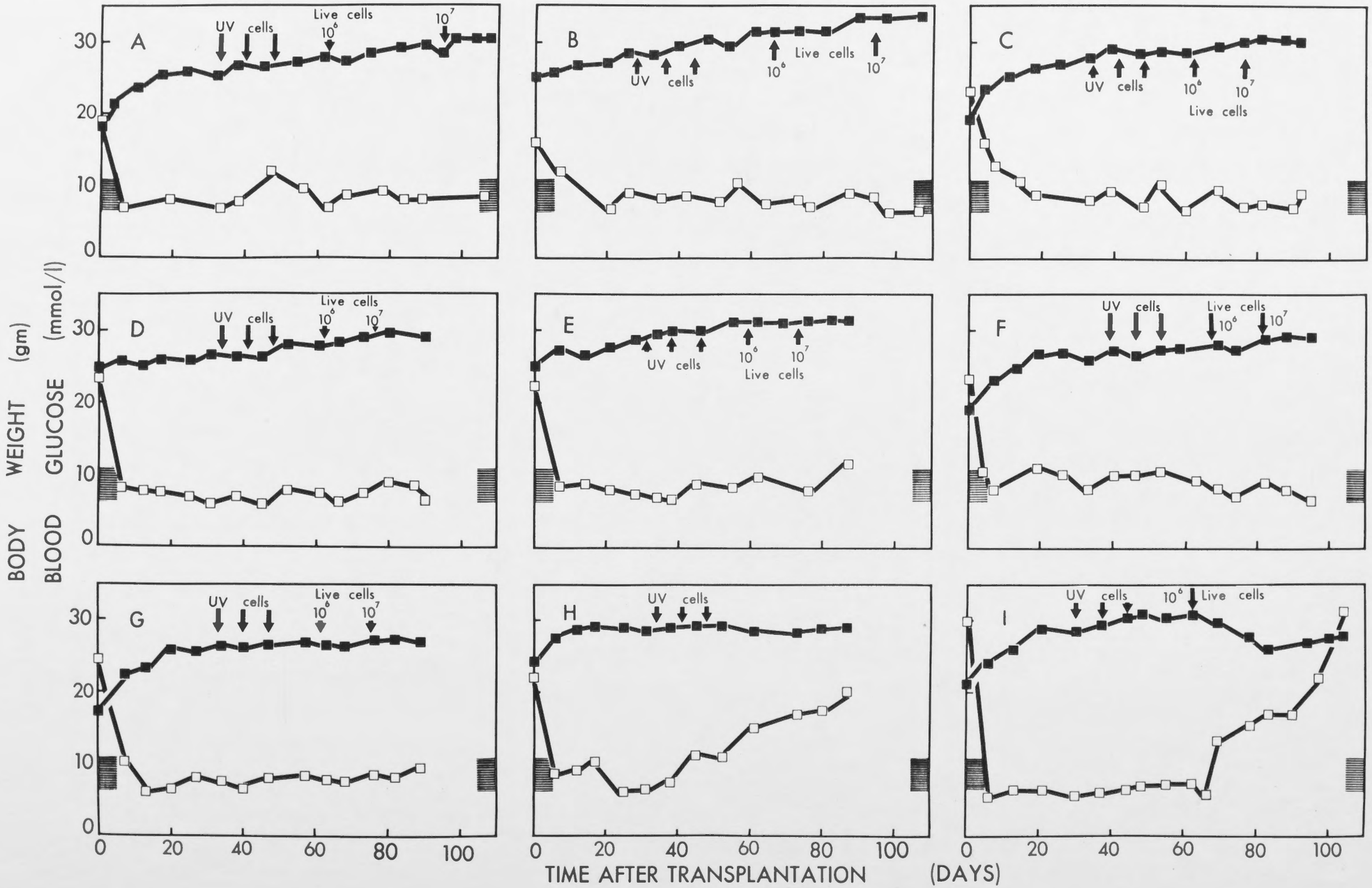
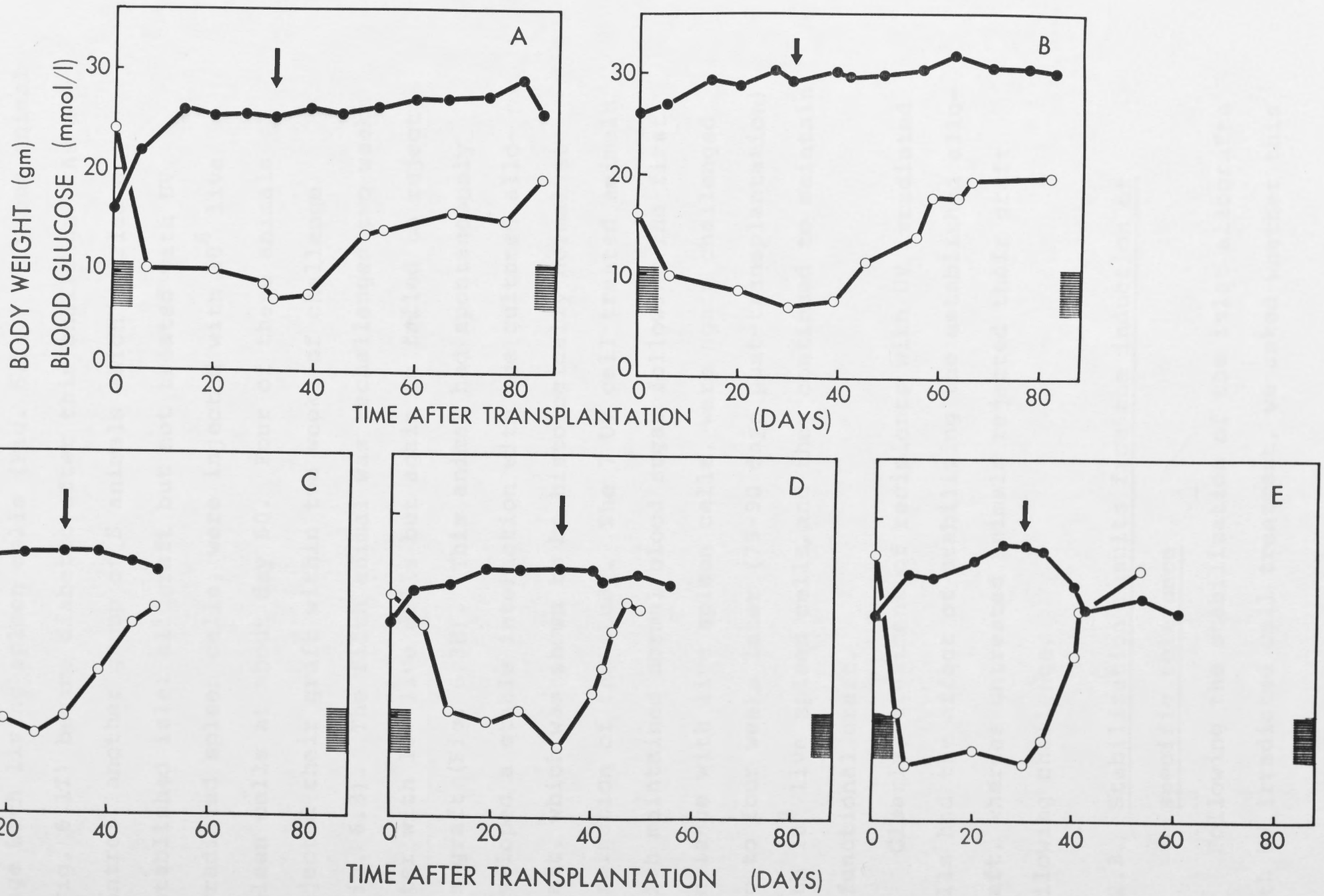

A
B
C
D
E
F
G
H
I
UV cells
Live cells
10^6
10^7
BODY WEIGHT (gm)
BLOOD GLUCOSE (mmol/l)
0
10
20
30
0
20
40
60
80
100
TIME AFTER TRANSPLANTATION (DAYS)

Figure 6.2

Non-fasting blood glucose levels (O) and body weight (●) of 5 CBA mice transplanted with 350 cultured allogeneic (BALB/c) islets and challenged with 10^6 live cells of donor origin at 30-34 days post transplantation.


A
B
C
D
E
BODY WEIGHT (gm)
BLOOD GLUCOSE (mmol/l)
0
10
20
30
0
20
40
60
80
TIME AFTER TRANSPLANTATION (DAYS)

ted cells (about 60 days post-transplantation), with 10^6 live spleen cells of donor origin (Fig. 6.1). Seven out of 8 animals maintained normal blood glucose levels and a corresponding increase in body weight following the challenge with living spleen cells (Fig. 6.1). Only one animal (Fig. 6.1I) became diabetic after this challenge. As a control, another group of 5 animals which carried an established islet allograft but not treated with UV irradiated spleen cells, were injected with 10^6 live spleen cells at about day 60. Four of these animals rejected their graft within two weeks of challenge (Fig. 6.3). The fifth animal was rechallenged two weeks later with 10^7 live cells but again it failed to reject its graft (Fig. 6.3E). This animal had spontaneously developed a stable interaction with the cultured allograft, which was shown to be histologically normal at termination of the study. The 7 UV cell treated animals which maintained normal blood sugar following the first challenge with live spleen cells, were again challenged two to four weeks later (75-90 days post-transplantation) with 10^7 live spleen cells, and they continued to maintain a functional graft.

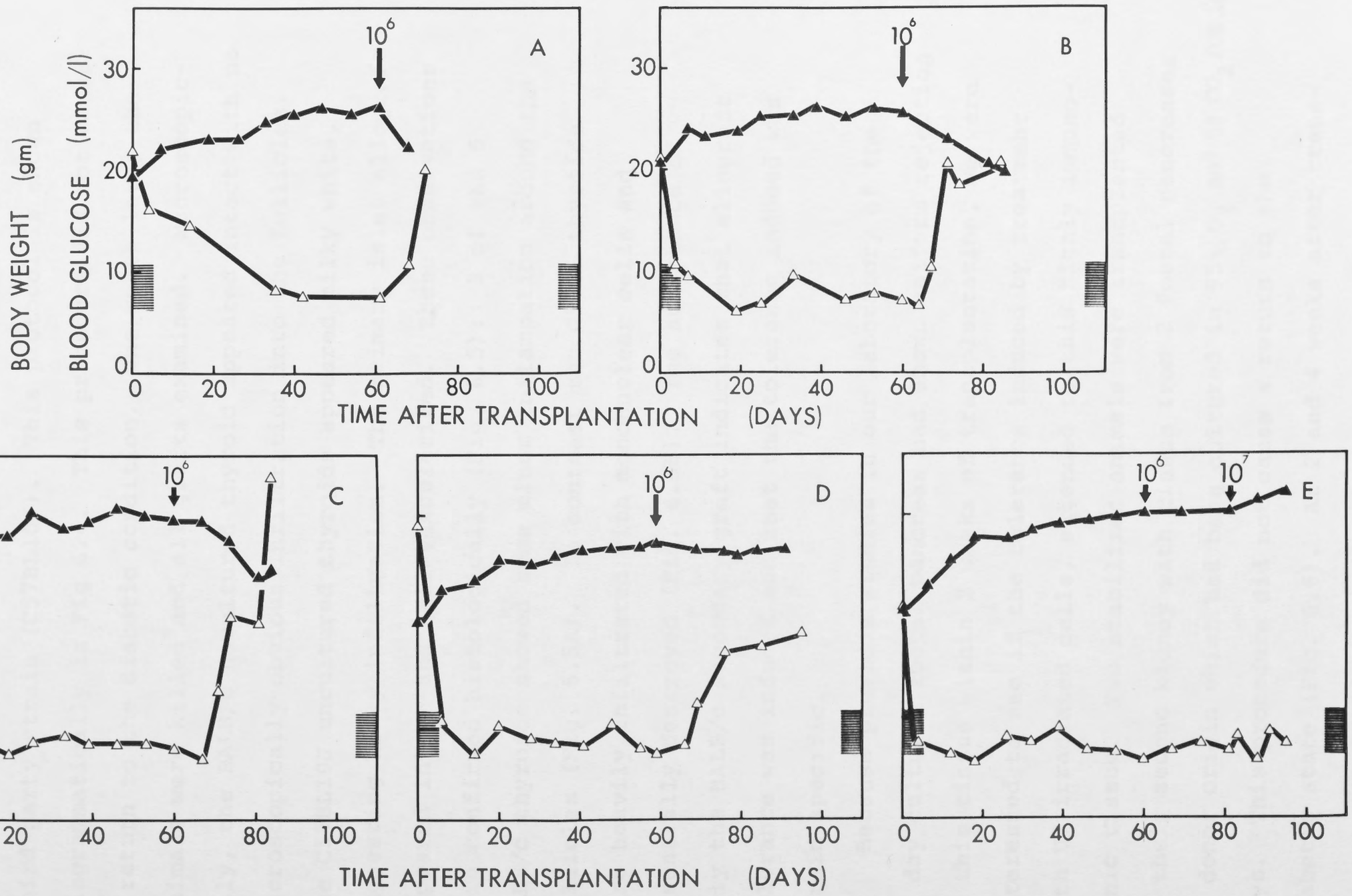
Clearly, treatment of recipients with UV irradiated cells had the effect of stabilizing the established allograft, whereas untreated animals rejected their graft following challenge.

6.2.3. Stabilization results from the induction of specific tolerance

Following the stabilization of the islet allografts with UV irradiated cell treatment, we asked whether this

Figure 6.3

Non-fasting blood glucose levels (Δ) and body weight (▲) of 5 CBA mice transplanted with 350 cultured allogeneic (BALB/c) islets and challenged with 10^6 live cells of donor origin at 56-61 days post transplantation. Only 1 animal (E) did not reject its graft after challenge.

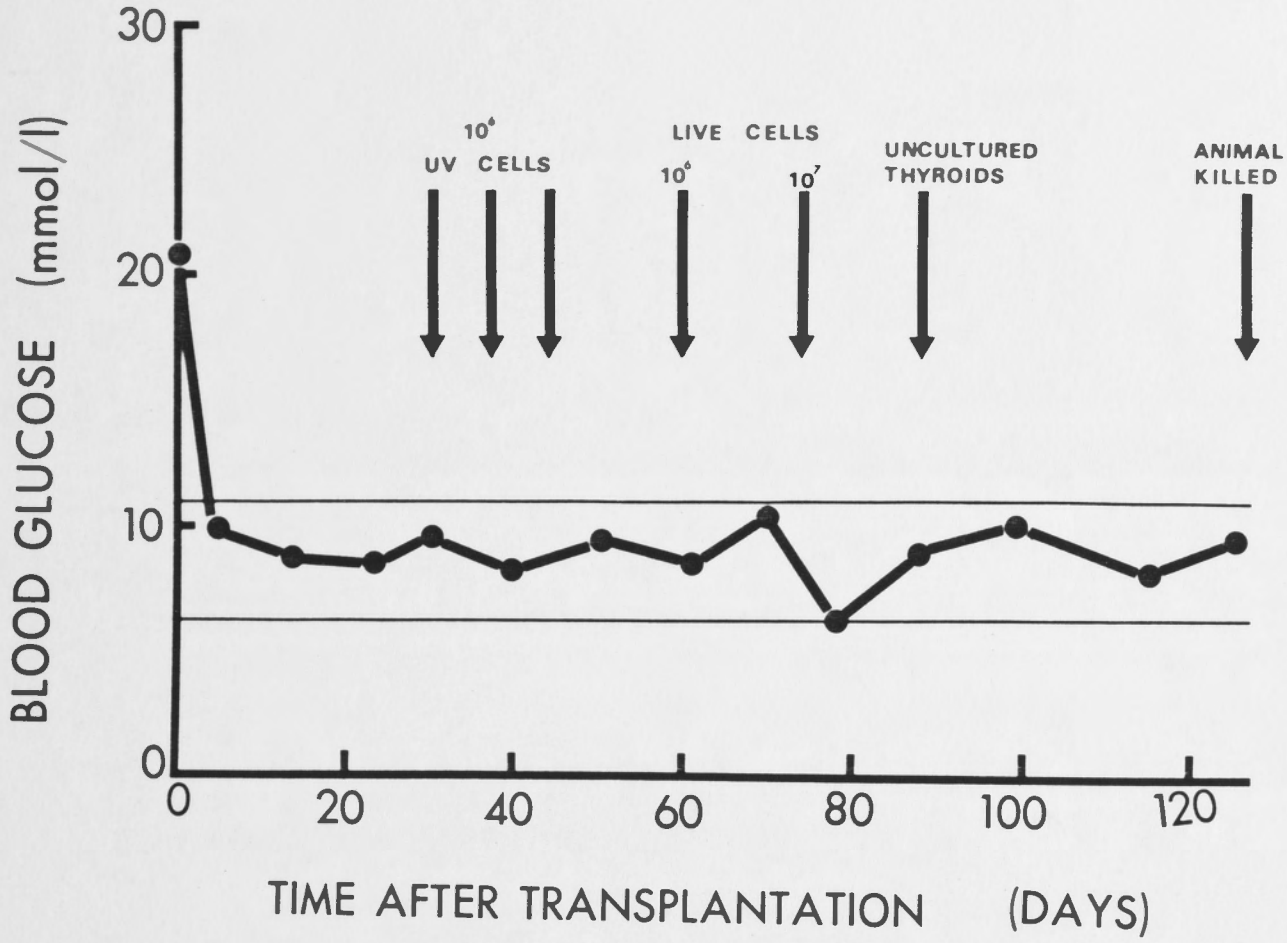

BODY WEIGHT (gm)
BLOOD GLUCOSE (mmol/l)
10^6
A
B
C
D
10^6
10^7
E
0
10
20
30
0
20
40
60
80
100
TIME AFTER TRANSPLANTATION (DAYS)

stability might be due to the induction of specific tolerance. To test this proposition, 5 animals carrying stabilized allografts were transplanted in their opposite kidney with uncultured thyroids of both donor origin (BALB/c) and from a third party strain (C57BL10J). This protocol is shown diagrammatically in Fig 6.4. This procedure did not cause a return to the diabetic condition. One month later the animals were killed and all grafts examined. Macroscopically, the BALB/c uncultured thyroid appeared intact with no macroscopically obvious infiltration into the follicles. The C57BL10J uncultured thyroids appeared milky white, suggesting heavy infiltration. The primary islet allograft appeared intact and well vascularized. These observations were confirmed histologically (Fig. 6.5); 3 of the 5 BALB/c thyroids showed some minor inflammation around the follicles (Fig. 6.5A). In contrast the C57BL thyroids were heavily infiltrated with mononuclear cells and essentially destroyed (Fig. 6.5B). The acceptance of only the BALB/c secondary graft indicates that allograft tolerance was induced and that the tolerance induced was strain specific.

Because previous studies in our laboratory of the 10 day cultured foetal pancreas had shown uniform rejection of this tissue within 2 weeks of transplantation, we were interested to see if the tolerance induced by treatment with UV irradiated cells, extended to this highly immunogenic tissue. Two stabilized animals were transplanted in their second kidney with tissue from 2 foetal pancreases of donor origin which had been cultured in 95% O_2 and 5% CO_2 for 10 days. This procedure did not cause a return to the diabetic state (Fig. 6.6). At 2 and 4 weeks after trans-

Figure 6.4

Diagrammatic representation of the protocol used to stabilize mouse islet allografts and to test the specificity of tolerance using uncultured thyroid of donor (BALB/c; H-2^d) and third party (C57BL10J; H-2^b) origin.


30
20
10
0
BLOOD GLUCOSE (mmol/l)
10⁶
UV CELLS
LIVE CELLS
10⁶
10⁷
UNCULTURED
THYROIDS
ANIMAL
KILLED
0
20
40
60
80
100
120
TIME AFTER TRANSPLANTATION (DAYS)

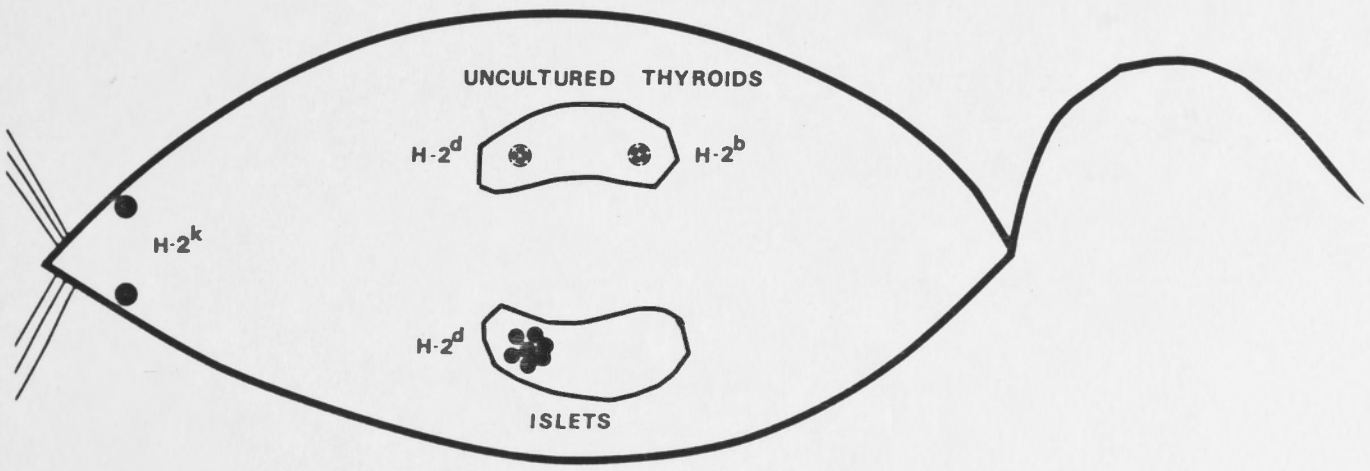

UNCULTURED THYROIDS
H-2d
H-2b
H-2k
H-2d
ISLETS

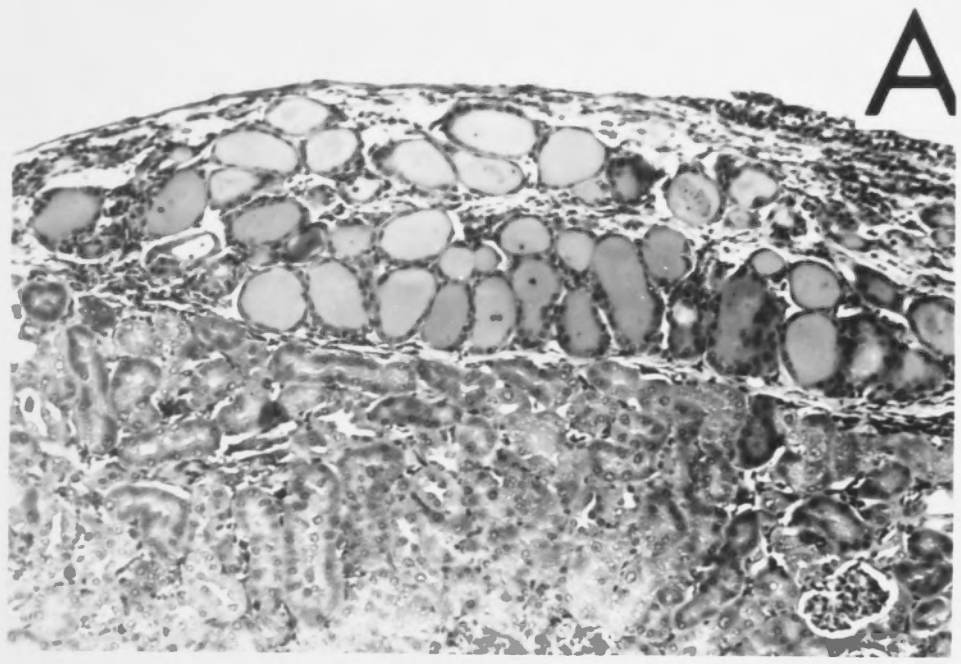
Figure 6.5

Histological appearance of primary and secondary allografts in a stabilized CBA recipient.

A. BALB/c uncultured thyroid. Note the presence of intact thyroid follicles.

B. C57BL10J uncultured thyroid showing a vigorous allograft response with thyroid tissue essentially destroyed.

C. BALB/c cultured islet tissue - the primary allograft. (haematoxylin and eosin, x 109).


A


B

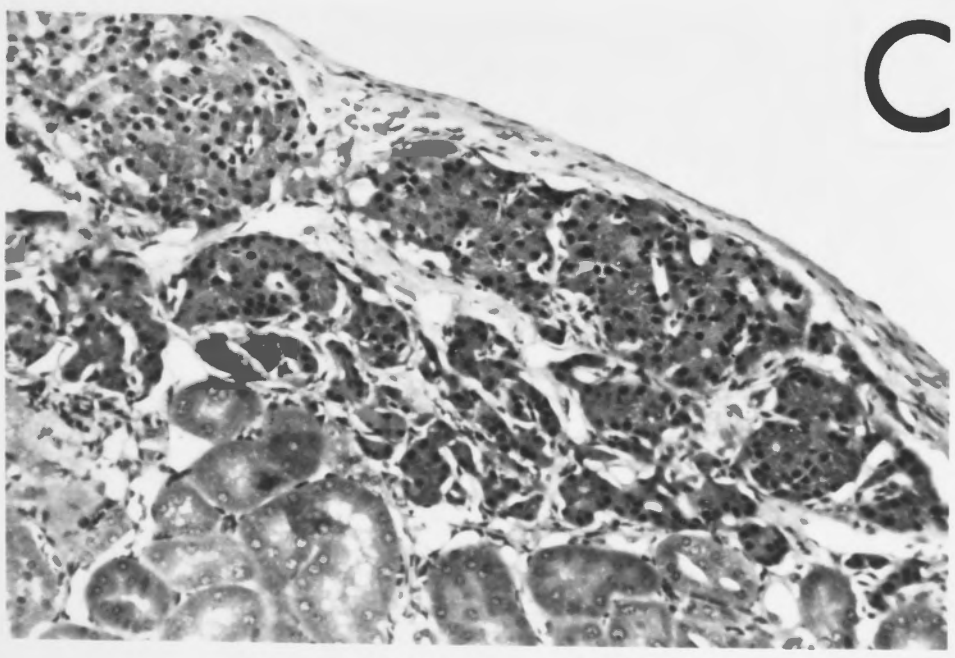
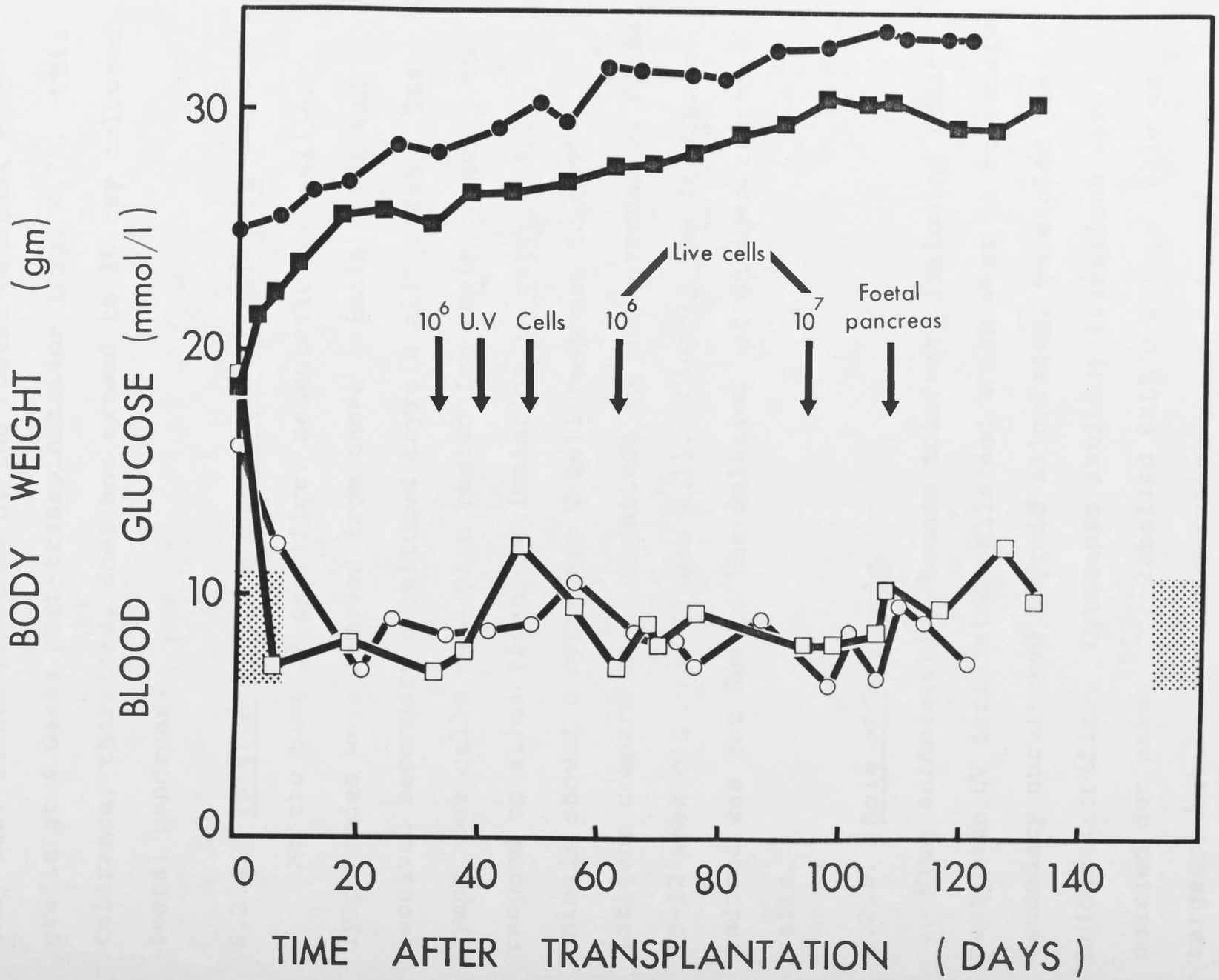

C

Figure 6.6

Non-fasting blood glucose levels (O,□) and body weight (●,■) of 2 stabilized CBA mice challenged with 10^6 and 10^7 live spleen cells of donor origin, and then given a second allograft of 10 day cultured foetal pancreas syngeneic with the primary islet allograft.


BODY WEIGHT (gm)
BLOOD GLUCOSE (mmol/l)
30
20
10
0
Live cells
10^6 U.V Cells
10^6
10^7
Foetal pancreas
0
20
40
60
80
100
120
140
TIME AFTER TRANSPLANTATION (DAYS)

plantation of the foetal pancreas, animals were sacrificed and both the primary and secondary grafts were examined. While the original graft of adult islets remained intact, only scar tissue remained of the foetal pancreas allografts at 4 weeks post-transplantation (Fig. 6.7). This tolerance, therefore, does not extend to 10 day cultured foetal pancreas.

6.2.4. Tolerance is not due to clonal deletion

At the time of sacrifice, mesenteric and axillary lymph nodes were removed from those animals which had received secondary uncultured thyroid allografts. The lymph node cells were then tested for their capacity to respond to alloantigenic stimulation *in vitro*. All animals showed a cytotoxic T cell response to H-2^d antigens comparable in magnitude to the response of normal 10-12 week old mice (Table 6.1). Clearly the tolerance induced was not due to the deletion of antigen reactive cells.

6.2.5. Role of antibody

Five animals which became tolerant following treatment with UV irradiated cells and which went on to receive secondary uncultured thyroid allografts, were bled just before sacrifice. Cytotoxic antibody titrations were carried out using ^{51}Cr-labelled BALB/c spleen cells as targets, but no cytotoxic antibody was detected.

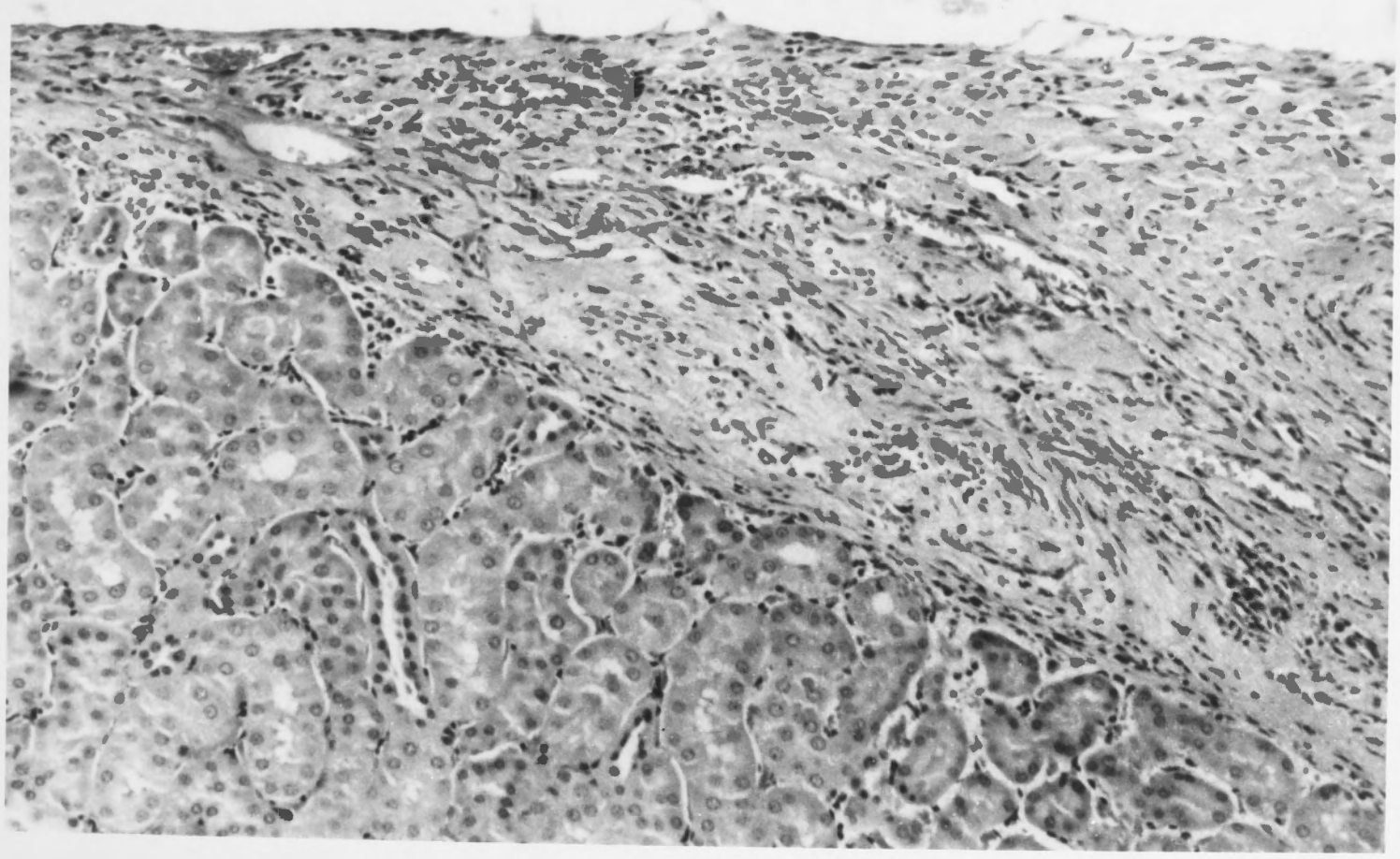


Figure 6.7

Histological appearance of 10 day cultured foetal BALB/c pancreas 2 weeks after transplantation beneath the kidney capsule of a CBA recipient carrying an established BALB/c islet allograft. Note the presence of rejection response (haematoxylin and eosin, x 164).

Table 6.1

Log_{10}Cytotoxic units (CU) per culture

	Grafted Animal	Control
	5.6	5.6
	5.3	5.3
	5.1	4.7
	4.8	4.7
	5.0	5.3
Mean ± S.D.	5.2 ± 0.3	5.1 ± 0.4

6.3 DISCUSSION

The results of this study demonstrate that there is a marked difference in immunogenicity between UV irradiated and live spleen cells. Treatment with UV irradiated cells, with one exception, did not induce the rejection of the islet allograft, whereas live cells injected at the same time following transplantation, rapidly destroyed the graft. This is consistent with the finding that UV irradiated cells are unable to stimulate allogeneic T cells *in vitro* (Lafferty *et al.* 1974). We are not sure why one animal out of nine rejected its graft after treatment with UV irradiated cells although it could have been the result of a technical error. A different UV irradiation source was used for treating cells for that particular animal, and as a result, some cells may have escaped killing. It is known that as few as 10^3 viable lymphocytes are capable of leading to allograft rejection (Lafferty *et al.* 1976b; Talmage *et al.* 1976).

The treatment of islet recipients with UV irradiated spleen cells of donor origin led to the stabilization of the islet allograft by inducing a state of tolerance. Animals treated in this way were, in all but one case, able to withstand subsequent challenge with 10^6 and 10^7 live spleen cells. Moreover, the acceptance of uncultured thyroids of donor origin by stabilized recipients, but not thyroids from a third party strain, illustrates the specificity of the tolerance induced. It should be noted, however, that H-2^d uncultured thyroids that were transplanted to animals whose islet graft had been stabilized, showed minor inflammation around the follicles.

While we cannot be certain of the mechanism underlying this reactivity, this response may be due to antigenic differences between cultured and uncultured tissue. Animals tolerant of the cultured islet allograft, from which Ia (class II) antigens have been eliminated during organ culture prior to grafting (Parr et al. 1980), might not be expected to be tolerant of class II antigens carried on the surface of leucocytes in the uncultured thyroid.

While the tolerance induced enabled recipients to maintain an uncultured thyroid allograft of donor origin, it was not sufficient to allow the survival of 10 day cultured foetal pancreas of similar origin. At 2 weeks this tissue was totally destroyed. We suggest that the rejection of the cultured foetal pancreas may be due to the existence in that tissue, despite its culture, of sufficient residual class II positive lymphoid cells which can lead to non-specific graft destruction via an inflammatory reaction. When explanted, foetal tissue is known to carry a lymphoid component which acts to supplement the population of blood cells carried as passengers within the tissue vasculature (Simeonovic et al. 1980). Human foetal pancreas has also been shown to carry numerous DR-positive (class II) lymphoid cells and dendritic cells (Danilovs et al. 1982). This large component makes the mouse foetal pancreas difficult for preparation for transplantation using organ culture (Simeonovic et al. 1980, 1983). In contrast, this is not a problem for thyroid or islet tissue; the latter being relatively purified from the lymphoid component by the hand-picking procedure (Section 2.3.1). Regarding the inflammatory

response to class II MHC antigens in the foetal pancreas, there are two ways this might occur. In addition to lymphokine secretion (following antigen recognition by activated host T cells) which then activates a host inflammatory response by the migration, activation and proliferation of host cells, the lymphokines can also act on donor (that is, of foetal pancreas origin) responsive blood cells causing them to proliferate and lead to non-specific damage. Both potential mechanisms could contribute to graft destruction. Thus, while the degree of class II contamination is only sufficient to cause a light infiltration into the uncultured thyroid tissue, in the foetal pancreas the class II component is sufficient to lead to destruction via an inflammatory response. This difference in size of class II antigen component may also account for the findings of White et al. (1980). They investigated the stability of established grafts, and found that where a heart was the primary established allograft, a secondary skin allograft given a long time after the withdrawal of CyA immunosuppression, was rejected. In contrast, the primary heart allograft was retained. Again, class II region mediated reactivity could account for this effect if class II bearing cells are eliminated from the heart allografts during the period of immunosuppression. Yet, where skin was the primary established graft, withdrawal of CyA resulted in its rejection; a secondary donor specific graft led to the rejection of both skin and the secondary graft. This may indicate that CyA was not able to eliminate all class II bearing cells in skin - a tissue

highly positive for class II antigen (Hammerling, 1976).

As already alluded above, this class II region difference may be reflected in differences in culture period requirements for graft survival. In our laboratory, mouse pancreatic islets can be successfully allografted after only 7 days in culture (Bowen et al. 1980), whereas foetal pancreas is consistently rejected after 7 and 10 days culture, and requires 17-20 days for significant survival (Simeonovic et al. 1980, 1983) and is functional at 20 days culture (Simeonovic and Lafferty, 1981). Thyroid allografts can survive with 12 days culture (Lafferty et al. 1975) although we routinely culture for 21 days.

The treatment of recipients with UV irradiated cells of donor origin is, therefore, able to markedly reduce the period of graft vulnerability by the induction of specific tolerance. Functional tolerance will ultimately develop in a proportion of animals carrying a cultured allograft (Bowen et al. 1981; Donohoe et al. 1983) although the period required to induce this tolerance can be as long as 350 days. Nagao et al. (1982) have also shown this development of tolerance with the passage of time in rats carrying heart allografts and immune suppresssed with CyA. Following the withdrawal of CyA two weeks after the heart allograft, they found that the heart continues to function but can be rejected if the recipient is challenged with donor skin; at this stage the heart allograft is in a metastable condition. With the further passage of time, at over 100 days, the heart graft was not affected

and skin survival was prolonged; the latter graft now undergoing a chronic rather than an acute rejection.

The mechanism of tolerance induction in these allografted animals is not known. The classical view of transplantation tolerance is one based on the clonal elimination of reacting cells (Burnet, 1959; Section 1.14). However, both in the case of thyroid (Donohoe et al. 1983) and in the present study with islets, we have shown that this phenomenon is not a deletion form of tolerance. In both models, lymphocytes from tolerant animals respond normally to donor alloantigen in vitro. In the case of thyroids, tolerant animals are hyporesponsive to in vivo challenge with donor cells (Donohoe et al. 1983). We have suggested that the slow, continued administration of alloantigen, by way of UV irradiated cells, might induce a state of tolerance in a manner somewhat similar to active enhancement (Donohoe et al. 1983). According to the original two signal model for lymphocyte activation, provision of antigen (signal 1) alone is tolerogenic (Bretscher and Cohn, 1970). UV treated cells are an effective source of signal 1; antigen is left intact on the cell surface of the irradiated cell (Lafferty et al. 1974; Talmage et al. 1977).

The mechanism by which enhancing antibodies prolong allograft survival is unresolved (Section 1.13.6). However, the idea that antigen-antibody complexes might be important in preventing rejection of allografts, gained some support with the work of the Hellstroms (reviewed by Hellstrom & Hellstrom, 1974; Section 1.14.2).

Hutchinson (1980) proposed that antigen reactive cells are opsonized by graft antigen-antibody complexes and are subsequently destroyed by Fc-receptor bearing host cells such as macrophages (Section 1.13.6.1). We have not been able to detect cytotoxic antibody, although this does not necessarily preclude its existence.

Lacy's group have suggested such tolerance might be due to the action of suppressor cells (Faustman et al. 1981, 1982a,b; Zitron et al. 1981b). T suppressor cells as an active mechanism in the maintenance of transplantation tolerance has been increasingly demonstrated and is discussed in Chapter 1 (Section 1.14.4). UV irradiation is known to impair immune responses (reviewed by Fox et al. 1980) and Greene et al. (1979) using UV irradiated mice, were able to demonstrate defective antigen-presenting cells in their spleens for both DTH and antibody responses. But whether the induction of suppressor cells can be accounted for by abnormalities in antigen presenting cells is not quite clear, although the evidence from Greene's group suggests that inappropriate presentation of antigen may lead to suppression and that the suppression can be transferred with T cells. Priming for DTH responses with antigen presenting cells from UV treated mice gave rise to suppressor T cells (Greene et al. 1979) and more recently, in a study using in vivo irradiated spleen cells, they again suggested that antigen presented on such cells could generate suppressors by the demonstration that such cells could suppress DTH responses (Fox et al. 1981).

We have shown, therefore, that in the mouse pancreatic islet model, the metastable condition of the graft following transplantation can be eliminated by treatment of the recipient with UV irradiated spleen cells of donor origin. It would be of interest to determine whether UV irradiated cells on their own are tolerogenic. That is, can pre-treatment of the recipient with UV irradiated cells followed by an uncultured allograft, lead to the acceptance of that graft? Attempts to transplant uncultured isolated pancreatic islets under the kidney capsule proved technically difficult because of the unclustered nature of the islets. However, some preliminary work in our laboratory using uncultured thyroids (unpublished data) is encouraging. Histological data show that pretreatment of the recipient with UV irradiated cells is definitely beneficial to the graft when compared to untreated controls. The UV cell dosage, however, seems to be of critical importance and further work is required to establish the optimum antigen dose and time course of treatment. Lau et al. (1983) very recently demonstrated that prolonged islet allograft survival in rats could be achieved by treating recipients with whole donor blood which had first been UV irradiated.

Our demonstration in the mouse pancreatic islet model that a state of specific tolerance can be induced in adult animals without a requirement for recipient immunosuppression, has potential for clinical transplantation where grafts are constantly under the threat of rejection.

6.4 SUMMARY

A comparison of the capacity of UV irradiated and live spleen cells of donor origin to induce islet allograft rejection showed that UV irradiated spleen cells were not immunogenic; live spleen cells were immunogenic and their injection triggered allograft rejection. Following treatment with irradiated spleen cells from about day 30 post-transplantation, recipient animals were able to withstand subsequent challenges with 10^6 and 10^7 viable donor spleen cells. Untreated animals rejected their graft when challenged with 10^6 donor spleen cells. That is, treatment with UV irradiated cells stabilized the islet allograft by inducing a state of tolerance. Subsequent transplantation of stabilized animals with uncultured thyroids of both donor and third party origin, demonstrated that the tolerance was specific. *In vitro* test of immune reactivity showed this tolerance was not due to the deletion of antigen reactive cells.

CHAPTER 7

GENERAL DISCUSSION

This thesis was primarily concerned with determining the vulnerability of established islet and thyroid allografts to rejection. While cultured allografts can be transplanted without a requirement for suppressing the recipient's immune system, such allografts are constantly under the threat of rejection. Clinically, this could have great importance. For example, a blood transfusion given for some therapeutic reason unconnected with the transplant could trigger rejection of the allograft if the blood cells carried MHC antigens common to those in the graft. The experimental work described in this report was, therefore, basically an investigation of potential threats to the established allograft, and the cellular requirements for its rejection. Furthermore, the possibility of rendering such grafts stable, was also investigated. This stabilization was achieved in recipients of islet allografts and was found to be due to the induction of specific tolerance - a finding which is most encouraging both from the basic immunobiological and clinical viewpoints.

Using in vitro culture to modulate tissue immunogenicity, islet and thyroid allografts were successfully transplanted across an MHC barrier without the use of immunosuppression. Such grafts are functional and are able to reverse diabetes or take up labelled iodine. This procedure of organ culture is one which is based on the theory that donor leucocytes carried within the graft initiate the rejection process (Section 1.7). The success achieved by using this technique, has potential clinical relevance in the treatment of insulin

dependent diabetes and its many degenerative complications.

Challenge of animals carrying islet and thyroid allografts with donor specific antibody and complement, was not able to lead to a rejection of the graft. This resistance is thought to be due to a revascularization of the graft tissue with host vascular endothelium. Revascularization occurs because culture of tissue results in the degeneration and loss of its own vascular endothelium (Parr et al. 1980b) - a loss which was thought by Hirschberg and coworkers (Section 1.7.2) to account for the reduction in immunogenicity. That is, they proposed that vascular endothelial cells have a stimulatory capacity in allograft rejection. However, there is now considerable evidence which suggests that reduction in immunogenicity following organ culture, is not due to the loss of vascular endothelium but, rather, to the inactivation or elimination of passenger leucocytes (Section 1.7.2). Such revascularization, however, is not able to protect the transplant from cell mediated damage. Challenge with cells of donor origin will lead to the prompt rejection of the allograft (Lafferty et al. 1976b; Talmage et al. 1976; Lafferty and Woolnough, 1977; Simeonovic et al. 1980; Vesole et al. 1982; Lacy et al. 1979c). That is, the cultured tissue remains antigenic and can be rejected once the recipient is activated with leucocytes of donor origin.

Other work from our laboratory has shown that cultured islet allografts are also resistant to non-specific stimulation of the host's immune response mechanisms (Bowen et al. 1981). Mice carrying established

allografts were able to resist challenge with Freund's complete adjuvant. This is also an important finding because the mycobacterial component of the adjuvant can stimulate the release of interleukin I from macrophages (Togawa et al. 1978). That is, non-specific stimulation could raise CoS activity levels and consequently lead to rejection of the graft. This finding suggests that cultured allografts would probably be resistant to other non-specific stimulation, such as infections.

In Chapters 4 and 5 we examined the cellular requirements and mode of action of these cells in the rejection of islet allografts. Such grafts are acutely rejected by sensitised cells of the Lyt 1^+ 2^+ subclass. Loveland et al. (1981) concluded that Lyt 1^+ 2^- T cells from sensitised mice could trigger skin graft rejection in ATXBM mice and concluded that cytotoxic cells (Lyt 1^+2^+) were not involved in allograft rejection. Our findings, however, would indicate that their conclusion cannot generally hold. We attempted to reconcile this conflict by suggesting that the subclass of T cell responsible for triggering graft rejection, probably depends on the type of tissue grafted which, in turn, is a reflection of the class of alloantigen expressed on that tissue.

The acute rejection of islet allografts following the transfer of sensitised cells, was initially thought to be consistent with a direct cytotoxic effect by the immune cells, although rejection via lymphokine release was not excluded. To answer this question, we used the immunosuppressive drug, CyA, in this case, as an analytical tool. CyA is known to inhibit the release of lymphokine

without affecting the cell's cytotoxic capacity. Our results indicate that lymphokine release plays an important role in the triggering of islet allograft rejection by Lyt $1^{+}2^{+}$ cells. These findings are also consistent with other work, both *in vivo* and *in vitro*, from our laboratory, and with virus elimination (Schiltknecht and Ada - personal communication). An interesting aspect of CyA treatment is that recovery can occur once the drug is withdrawn.

Earlier work from our laboratory had produced the unexpected finding that functional tolerance can develop in recipients of long-standing islet and thyroid allografts (Bowen *et al*. 1981; Donohoe *et al*. 1983). Such recipients are resistant to challenge with peritoneal or spleen cells of donor origin. However, until such tolerance develops, which can take from 3 to 12 months depending on the type of tissue, the graft remains in a metastable phase. We extended these studies, with our initial aim being to try and reduce the metastable period. We attempted to do this by treatment of the host with antigen alone given in the form of donor spleen cells which had been killed by UV irradiation. It has already been demonstrated that transplantation antigen is highly immunogenic when presented to T cells on the surface of metabolically active cells which also provide a source or CoS activity; these are the S^{+} cells (Section 1.6.2). The metabolic inactivation of these cells with UV irradiation destroys their capacity to activate a cytotoxic T cell response (Lafferty *et al*. 1983a). We now demonstrated that treatment of islet allograft recipients with UV irradiated donor spleen cells

does indeed bring on a situation of graft stability - such recipients can now withstand challenge with 10^6 and 10^7 live spleen cells of donor type. The treatment with UV irradiated cells induced a state of specific tolerance - such recipients were now able to accept uncultured thyroid grafts of donor origin. This evidence is confirmatory of the 2 signal model for lymphocyte activation proposed by Bretscher and Cohn (1970) and Lafferty and coworkers (Section 1.6.2), which predicts that presentation of antigen in a non-immunogenic form is tolerogenic. Support for this view has also come from Faustman et al. (1982a,b) who were able to obtain prolonged islet allograft survival following preimmunization of mice with donor specific blood depleted of class II antigen bearing cells. More recently, Lau et al. (1983) achieved the same effect by treating recipients with whole donor blood which had first been UV irradiated. Clinically, our findings are also important because they demonstrate the possibility of inducing allograft tolerance. Such treatment could potentially not only offer protection against the initiation of graft rejection following transplantation, but also give protection later in a situation where the patient might be exposed to cross reacting antigens such as might occur during a blood transfusion. Such immunological manipulation of the recipient is further encouraging clinically, because it can produce a protective effect without harming the recipient by reducing his immunity to infection - a problem long recognized with drug immunosuppression (Section 1.12.1).

One question which remains to be answered is the mechanism of this tolerance. Although this phenomenon is

not due to a deletion form of tolerance, since we showed that lymphocytes from such tolerized animals respond normally to donor alloantigens in vitro, further work is required to elucidate the possible mechanism(s). Although we have no firm evidence concerning the nature of this tolerance or how it is induced, it would seem that the way the antigen is delivered to the immune system affects its response. We have proposed, therefore, that this form of tolerance might develop as a result of the slow liberation of antigen into the host's immune system which could then lead to tolerance induction in a way somewhat similar to active enhancement (Lafferty et al. 1983a). The mechanisms responsible for the induction and maintenance of immunological enhancement are still subject to speculation and controversy (Section 1.13.6). However, of the several mechanisms proposed, there is little support for clonal deletion, with the more favoured explanations being the induction of antiidiotypic antibodies and suppresssor cells. Mechanisms for transplantation tolerance are discussed in Section 1.14. In view of the increasing evidence for the role of T suppressor cells (Dorsch and Roser, 1977; Hilgert, 1979; Tutschka et al. 1981a,b; Roser et al. 1983; Section 1.14.4), further work would be required in the cultured islet allograft model to investigate their possible role in this system. Work along these lines by Lacy and his coworkers (Zitron et al. 1981b) have already implicated suppressor cell activity in rats carrying long-term islet allografts. Further work is also required to determine if the tolerance state can be transferred to a naive animal of recipient genotype and

the effect UV irradiation has on antigen presenting cells. Such studies by Benacerraf's group (Greene et al. 1979; Fox et al. 1980, 1981) suggest that antigen presentation by such cells is affected and this may lead to the induction of suppressor cells. Because of the potential these findings hold for clinical transplantation in general, more information concerning the nature of the antigens that induce this tolerance is also required.

Finally, the results presented in this report have demonstrated that cultured allografts, although they can be successfully transplanted across MHC barriers without the use of immunosuppression, still retain their antigenicity. That is, they remain susceptible to rejection for some time after transplantation. The cellular requirements for their demise are T cells of the Lyt 1^+2^+ or cytotoxic subclass and these can trigger rejection by lymphokine release. Of some interest has been the finding that such metastable grafts can in fact be stabilized by the induction of specific tolerance. The mechanism of this tolerance seems to be due to suppression, by an unknown mechanism, of the process of recipient sensitisation. Many other questions, unfortunately, remain unanswered and await further investigation. From these findings, and the review of the literature in Chapter 1, it is clear that the field of organ transplantation is one which, despite great progress, many problems still require resolution. Nonetheless, one of the most encouraging aspects of transplantation biology today, is the fact that there have probably never been so many tools with which to seek answers to the problems.

REFERENCES

Algire, G.H., Weaver, J.M. and Prehn, R.T. (1954). Growth of cells *in vivo* in diffusion chambers. I. Survival of homografts in immunized mice. J. Natl. Cancer Inst. 15: 493-507.

Algire, G.H., Weaver, J.M. and Prehn, R.T. (1957). Studies on tissue homotransplantation in mice using diffusion-chamber methods. Ann. N.Y. Acad. Sci. 64: 1009-1012.

Algire, G.H. (1959). Growth inhibition of homografts of a plasma cell neoplasm in cell-impenetrable diffusion chambers placed in hyperimmunized mice. J. Natl. Cancer Inst. 23: 435-439.

Amos, D.B. and Wakefield, J.D. (1959). Growth of mouse ascites tumour cells in diffusion chambers. II. Lysis and growth inhibition by diffusible isoantibody. J. Natl. Cancer Inst. 22: 1077-1092.

Anderson, D., Billingham, R.E., Lampkin, G.H. and Medawar, P.B. (1951). The use of skin grafting to distinguish between monozygotic and dizygotic twins in cattle. Heredity, 5: 379-397.

Andersson, A. and Hellerstrom, C. (1972). Metabolic characteristics of isolated pancreatic islets in tissue culture. Diabetes, Suppl. 21: 546-554.

Andersson, A. (1976). Tissue culture of isolated pancreatic islets. Acta Endocr. Copenh. Suppl. 205: 283-293.

Andrus, L. and Lafferty, K.J. (1980). Cellular requirements for production and release of the lymphocyte costimulator. Aust. J. Exp. Biol. Med. Sci. 58: 545-555.

Andrus, L., Prowse, S.J. and Lafferty, K.J. (1980). Interleukin 2 production by T cells: triggering of T cell activation and lymphokine release. Behring Inst. Mitt. 67: 61-67.

Andrus, L., Prowse, S.J. and Lafferty, K.J. (1981). Interleukin 2 production by both Ly 2^+ and Ly 2^- T-cell subsets. Scand. J. Immunol. 13: 297-301.

Andrus, L. and Lafferty, K.J. (1982). Inhibition of T-cell activity by Cyclosporin A. Scand. J. Immunol. 15: 449-458.

Archer, F.J. (1983). Monolayer culture of neonatal pig pancreatic islet cells. Diabetologia, 24: 185-190.

Ascher, N.L., Ferguson, R.M., Hoffman, R. and Simmons, R.L. (1979). Partial characterization of cytotoxic cells infiltrating sponge matrix allografts. Transplantation, 27: 254-259.

Ascher, N.L., Chen, S., Hoffman, R. and Simmons, R.L. (1931). Maturation of cytotoxic effector cells at the site of allograft rejection. Transplant. Proc. 13: 1105-1107.

Atkins, R.C. and Ford, W.L. (1972). The effect of lymphocytes and serum from tolerant rats on the graft-versus-host activity of normal lymphocytes. Transplantation, 13: 442-444.

Auchincloss, H. and Sachs, D.H. (1983). Mechanisms of tolerance in murine radiation bone marrow chimeras. II. Absence of nonspecific suppression in mature chimeras. Transplantation, 36: 442-445.

Bach, F.H. (1973). The major histocompatibility complex in transplantation immunology. Transplant. Proc. 5: 23-29.

Bach, F.H. and Van Rood, J.J. (1976). The major histocompatibility complex - genetics and biology. New Eng. J. Med. 295: 806-813; 872-878; 927-936.

Bach, F.H., Bach, M.L. and Sondel, P.M. (1976). Differential function of major histocompatibility complex antigens in T-lymphocyte activation. Nature, 259: 273-281.

Bach, F.H., Grillot-Courvalin, C., Kuperman, O.J., Sollinger, H.W., Hayes, C., Sondel, P.M., Alter, B.J. and Bach, M.L. (1977). Antigenic requirements for triggering of cytotoxic T lymphocytes. Immunological Rev. 35: 76-96.

Bach, F.H., and Alter, B.J. (1978). Alternative pathways of thymocyte activation. J. Exp. Med. 148: 829-834.

Ballinger, W.F. and Lacy, P.E. (1972). Transplantation of intact pancreatic islets in rats. Surgery, 72: 175-186.

Bansal, S.C., Hellstrom, I., Hellstrom, K.E. and Wright, P.W. (1973). Cell-mediated immunity and blocking serum activity before and after breakage of allograft tolerance in rats. Transplantation, 16: 610-620.

Barker, C.F. and Billingham, R.E. (1977). Immunologically privileged sites. Adv. Immunol. 25: 1-54.

Batchelor, J.R., Brent, L. and Kilshaw, P.J. (1977). Absence of suppressor cells from rats bearing passively enhanced kidney allografts. Nature, 270: 522-524.

Batchelor, J.R., Welsh, K.I. and Burgos, H. (1978). Transplantation of antigens per se are poor immunogens within a species. Nature, 273: 54-56.

Batchelor, J.R., Welsh, K.I., Maynard, A. and Burgos, H. (1979). Failure of long-surviving, passively enhanced kidney allografts to provoke T-dependent alloimmunity. I. Retransplantation of (ASxAUG)F_1 kidneys into secondary AS recipients. J. Exp. Med. 150: 455-464.

Batchelor, J.R. (1983). Donor-specific immunosuppression. Transplant. Proc. 15: 692-697.

Berden, J.H., Bogman, M.J., Hagemann, J.F., Tamboer, W.P. and Koene, R.A. (1981). Complement-dependent and independent mechanisms in acute antibody-mediated rejection of skin xenografts in the mouse. Transplantation, 32: 265-270.

Beveridge, T. (1983). Cyclosporin-A: an evaluation of clinical results. Transplant. Proc. 15: 433-437.

Billingham, R.E., Brent, L. and Medawar, P.B. (1953). 'Actively acquired tolerance' of foreign cells. Nature, 172: 603-606.

Billingham, R.E., Brent, L. and Medawar, P.B. (1954). Quantitative studies on tissue transplantation immunity. II. The origin, strength and duration of activity and adoptively acquired immunity. Proc. Roy. Soc. B. 143: 58-80.

Billingham, R.E. and Brent, L. (1956). Further attempts to transfer transplantation immunity by means of serum. Brit. J. Exp. Path. 37: 566-569.

Billingham, R.E., Brent, L. and Medawar, P.B. (1956a). Quantitative studies on tissue transplantation immunity. III. ACtively acquired tolerance. Phil. Trans. Roy. Soc. B. 239: 357-414.

Billingham, R.E., Brent, L. and Medawar, P.B. (1956b). 'Enhancement' in normal homografts, with a note on its possible mechanism. Transplantation Bull. 3: 84-88.

Billingham, R.E. and Brent, L. (1957). A simple method for inducing tolerance of skin homografts in mice. Transplantation Bull. 4: 67-71.

Billingham, R.E., Brent, L. and Medawar, P.B. (1958). Extraction of antigens causing transplantation immunity. Transplantation Bull. 5: 377-381.

Billingham, R.E. and Silvers, W.K. (1962). Studies on cheek pouch skin homografts in the Syrian hamster. In: Ciba Foundation Symposium on Transplantation. Wolstenholme, G.E. and Cameron, M.P. (eds). J.A. Churchill Ltd., London, pp. 90-117.

Billingham, R.E., Silvers, W.K. and Wilson, D.B. (1963). Further studies on adoptive transfer of sensitivity to skin homografts. J. Exp. Med. 118: 397-420.

Billingham, R.E. (1971). The passenger cell concept in transplantation immunology. Cell. Immunol. 2: 1-12.

Binz, H. and Wigzell, H. (1975). Shared idiotypic determinants on B and T lymphocytes reactive against the same antigenic determinants I. Demonstration of similar or identical idiotypes on IgG molecules and T cell receptors with specificity for the same alloantigens. J. Exp. Med. 142: 197-211.

Blanden, R.V. (1980). How do immune response genes work? Immunology Today, 1: 33-36.

Bogman, M.J., Berden, J.H.M., Hagemann, J.F.H., Maass, C.N. and Koene, R.A.P. (1980). Patterns of vascular damage in the antibody mediated rejection of skin xenografts in the mouse. Am. J. Path. 100: 727-735.

Bordes-Aznar, J., Lear, P.A., Strom, T.B., Tilney, N.L. and Kupiec-Weglinski, J.W. (1983). Kinetics of Cyclosporin-A-induced unresponsiveness to cardiac-allografts in rats. Transplant. Proc. 15: 500-503.

Borel, J.F. (1980). Immunosuppressive properties of Cyclosporin A (CY-A). Transplant. Proc. 12: 233.

Borel, J.F. (1981). From our laboratories Cyclosporin A. Triangle, 20: 97-105.

Borel, J.F. and Lafferty, K.J. (1983). Cyclosporine: speculation about its mechanism of action. Transplant. Proc. 15: 1881-1885.

Borleffs, J.C.C., Newhaus, P., Van Rood, J.J. and Balner, H. (1982). Platelet transfusions improve kidney allograft survival in rhesus monkeys without inducing cytotoxic antibodies. Lancet, 1: 1117-1118.

Bowen, K.M. and Lafferty, K.J. (1980). Reversal of diabetes by allogeneic islet transplantation without immunosuppression. Aust. J. Exp. Biol. Med. Sci. 58: 441-447.

Bowen, K.M., Andrus, L. and Lafferty, K.J. (1980). Successful allotransplantation of mouse pancreatic islets to nonimmunosuppresed recipients. Diabetes. 29: Suppl. 1 : 98-104.

Bowen, K.M., Prowse, S.J. and Lafferty, K.J. (1981). Reversal of diabetes by islet transplantation: vulnerability of the established graft. Science, 213: 1261-1262.

Boyd, W.C. (1966). Immunological tolerance and intolerance: Fate of tissue transplants (Chapter 12). In: Fundamentals of Immunology, 4th edition, Interscience Pubs. N.Y. pp. 507-520.

Boyse, E.A., Old, L.J. and Luell, S. (1963). Antigenic properties of experimental leukemias. II. Immunological studies in vivo with C57BL/6 radiation induced leukemias. J. Natl. Cancer Inst. 31: 987-995.

Brent, L. (1958). Tissue transplantation immunity. Progr. Allergy. 5: 271-348.

Brent, L. and Kilshaw, P.J. (1970). Prolongation of skin allograft survival with spleen extracts and anti-lymphocyte serum. Nature, 227: 898-900.

Brent, L., Hansen, J.A. and Kilshaw, P.J. (1971). Unresponsiveness to skin allografts induced by tissue extracts and antilymphocyte serum. Transplant. Proc. 3: 684-687.

Brent, L., Brooks, C., Lubling, N. and Thomas, A.V. (1972). Attempts to demonstrate an in vivo role for serum blocking factors in tolerant mice. Transplantation, 14: 382-387.

Brent, L., Hansen, J.A., Kilshaw, P.J. and Thomas, A.V. (1973). Specific unresponsiveness to skin allografts in mice. I. Properties of tissue extracts and their synergistic effect with antilymphocytic serum. Transplantation, 15: 160-171.

Brent, L., Brooks, C.G. Medawar, P.B. and Simpson, E. (1976). Transplantation tolerance. Brit. Med. Bull. 32: 101-106.

Brent, L. and Opara, S.C. (1979). Specific unresponsiveness to skin allografts in mice. V. Synergy between donor tissue extract, procarbazine hydrochloride and anti-lymphocyte serum in creating a long-lasting unresponsiveness mediated by suppressor T cells. Transplantation, 27: 120-126.

Brent, L., Bain. A.G., Butler, R., Horsburgh, T., Opara, S.C. and Wood, P.J. (1981). The antigenicity of purified liver parenchyma cells. Transplant. Proc. 13: 860-862.

Bretscher, P. and Cohn, M. (1970). A theory of self-noneself discrimination. Science, 169: 1042-1049.

Britton, S. and Palacios, R. (1982). Cyclosporin A-usefulness, risks and mechanism of action. Immunol. Rev. 65: 5-22.

Brooks, C.G. (1975). Neonatally induced transplantation tolerance: in vitro evidence supporting a clonal inactivation mechanism. Eur. J. Immunol. 5: 741-747.

Brooks, J.R., Sturgis, S.H. and Hill, G.J. (1960). An evaluation of endocrine tissue homo-transplantation in the millipore chamber: with a note on tissue adaptation to the host. Ann. N.Y. Acad. Sci. 87: 482-495.

Bunjes, D., Hardt, C., Rollinghoff, M. and Wagner, H. (1981). Cyclosporin A mediates immunosuppression of primary cytotoxic T cell responses by impairing the release of interleukin 1 and interleukin 2. Eur. J. Immunol. 11. 657-661.

Burdick, J.F., Russell, P.S. and Winn, H.J. (1979). Sensitivity of long-standing xenografts of rat hearts to humoral antibodies. J. Immunol. 123: 1732-1735.

Burnet, F.M. and Fenner, F. (1949). The Production of Antibodies. 2nd edition. Macmillan, Melbourne.

Burnet, F.M., Stone, J.D. and Edney, M. (1950). The failure of antibody production in the chick embryo. Aust. J. Exp. Biol. Med. Sci. 28: 291-297.

Burnet, M. (1959). The Clonal Selection Theory of Acquired Immunity. Cambridge Univ. Press, Cambridge.

Burnet, F.M. (1961). Immunological recognition of self. Science, 133: 307-311.

Bussolati, G. and Bassa, T. (1974). Thiosulfation aldehyde fuchsin (TAF) procedure for the staining of pancreatic B cells. Stain Tech. 49: 313-315.

Calne, R.Y., Davis, D.R., Medawar, P. and Wheeler, J.R. (1966). Effect of donor antigen on dogs with renal homotransplants. Transplantation, 4: 742-746.

Cannon, J.A. and Longmire, W.P. (1952). Studies of successful skin homografts in the chicken. Description of a method of grafting and its application as a technic of investigation. Ann. Surg. 135: 60-68.

Cannon, J.A. (1957). The question of host adaptation versus graft adaptation in successful homografts. Transplantation Bull. 4: 22-24.

Cantor, H. and Boyse, E.A. (1975). Functional subclass of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T-cell subclasses in a differentiative process independent of antigen. J. Exp. Med. 141: 1376-1389.

Cantor, H. and Boyse, E.A. (1976). Regulation of cellular and humoral immune responses by T-cell subclasses. Cold Spring Harbor Symposia on Quantitative Biology, 41: 23-32.

Carlson, G.A. and Terres, G. (1976). Antibody-induced killing in vivo of L1210/MTX-R cells quantitated in passively immunized mice with ^{132}I-iododeo-xyuridine labelled cells and whole-body measurements of retained radioactivity. J. Immunol. 117: 822-829.

Carpenter, C.B., D'Apice, A.J.F. and Abbas, A.K. (1976). The role of antibodies in the rejection and enhancement of organ allografts. Adv. Immunol. 22: 1-65.

Carpenter, C.B. (1981). The cellular basis of allograft rejection. Immunology Today, 2: 50-54.

Charpentier, B.M., Lang, P., Martin, B. and Fries, D. (1982). Specific recipient-donor unresponsiveness mediated by a suppressor cell system in human kidney allograft tolerance. Transplantation, 33: 470-477.

Chase, M.W. (1946). Inhibition of experimental drug allergy by prior feeding of the sensitizing agent. Proc. Soc. Exp. Biol. Med. 61: 257-259.

Chase, M.W. (1959). Immunologic tolerance. Ann. Rev. Micro. 13: 349-376.

Chavez-Peon, F., Monchik, G., Winn, H.T. and Russell, P.S. (1971). Humoral factors in experimental renal allograft and xenograft rejection. Transplant. Proc. 3: 573-576.

Chernyakmovskaya, I.Y., Nagurskaya, E.V., Shaposhnikova, G.B. Prigozhina, T.B. and Fontalin, L.N. (1980). Tolerance to allogeneic and to xenogeneic heart grafts provided by thymectomy of adult mice combined with donor cell and cyclophosphamide inoculation. Transplantation, 29: 409-412.

Chutna, J., Hasek, M., Sladecek, M., Viklicky, V. and Rubenik, J. (1973). Analysis of immunological reactivity in skin-allograft tolerating rats. Fol. Biol. 19: 252-260.

Cinader, B. and Dubert, J.M. (1955). Acquired immune tolerance to human albumin and the response to subsequent injection of diazo human albumin. Brit. J. Exp. Path. 36: 515-529.

Cochrum, K., Hanes, D., Potter, D., Perkins, H., Amend, W., Vincenti, F., Iwaki, Y., Opelz, G., Terasaki, P., Levin, B., Samason, D., Feduska, N. and Salvatierra, O. (1981). Improved graft survival following donor-specific blood transfusions. Transplant. Proc. 13: 1657-1660.

Corley, R.B. and Kindred, B. (1977). *In vivo* responses of alloreactive lymphocytes stimulate *in vitro*. Skin graft rejection mediated by MLR-primed lymphocytes. Scand. J. Immunol. 6: 991-996.

Cosimi, A.B., Burton, R.C., Colvin, R.B., Goldstein, G., Delmonico, F.L., La Quaglia, M.P., Tolkoffrubin, N., Rubin, R.H., Herrin, J.T. and Russell, P.S. (1981a). Treatment of acute renal allograft rejection with OKT3 monoclonal antibody. Transplantation. 32: 535-539.

Cosimi, A.B., Colvin, R.B., Burton, R.C., Rubin, R.H., Goldstein, G., Kung, P.C., Hansen, W.P., Delmonico, F.L. and Russell, P.S. (1981b). Use of monoclonal antibodies to T-cell subsets for immunologic monitoring and treatment in recipients of renal allografts. New Eng. J. Med. 305: 308-314.

Craighead, J.E. (1981). Viral diabetes mellitus in man and experimental animals. Amer. J. Med. 70: 127-134.

Curtain, C.C. (1959). The use of acquired immunological tolerance and immunological "paralysis" in the study of the antigenic relationships of normal and abnormal serum globulins. Brit. J. Exp. Path. 40: 255-262.

Dallman, M.J. and Mason, D.W. (1982) Role of thymus-derived and thymus-independent cells in immune skin allograft rejection. Transplantation, 33: 221-223.

Danilovs, J.A., Hofman, F.M., Taylor, C.R. and Brown, J. (1982) Expression of HLA-DR antigens in human fetal pancreas tissue. Diabetes, 31: (Suppl. 4) 23-28.

Davies, D.A.L., and Alkins, B.J. (1974). What abrogates heart transplant rejection in immunological enhancement? Nature, 247: 294-297.

Davies, D.A.L. and Staines, N.A. (1976). A cardinal role for I-region antigens (Ia) in immunological enhancement and the clinical implications. Transplant. Rev. 30: 18-39.

Davies, W.C. and McKenzie, I.F.C. (1980). Immunological enhancement can be mediated by anti-Ia and anti-K, D antibodies. Transplantation, 29: 77-79.

Day, E.D., Kaliss, N., Aronsen, A.I., Bryant, B.F., Friendly, D., Gabrielson, F.C. and Smith, P.M. (1954). Investigations of substances in mouse tissues inducing alteration of normal host-homograft relationships. J. Natl. Cancer. Inst. 15: 145-159.

Deeg, H.J., Storb, R., Weiden, P.L., Raff, R.F., Sale, G.E., Atkinson, K., Graham, T.C. and Thomas, E.D. (1982). Cyclosporin A and methotrexate in canine marrow transplantation: engraftment, graft-versus-host disease, and induction of tolerance. Transplantation, 34: 30-35.

Dennert, G. and Yamagata, S. (1980). Cloned cell lines of lymphocytes with natural and specific cytolytic activity. Behring Inst. Mitt. 67: 205-212.

De Sousa, M.A., Parrott, D.M. and Pantelouris, E.M. (1969). The lymphoid tissues in mice with congenital aplasia of the thymus. Clin. Exp. Immunol. 4: 637-644.

De Waal, R.M.W., Capel, P.J.A. and Koene, R.A.P. (1980). The role of anti-H-2K and H-2D allo-antibodies in engrafted and acute antibody-mediated rejection of mouse skin allografts. J. Immunol. 124: 719-723.

De Waal, R.M., Bogman, M.J., Maass, C.N., Cornelissen, L.M., Tax, W.J. and Koene, R.A. (1983). Variable expression of Ia antigens on the vascular endothelium of mouse skin allografts. Nature, 303: 426-429.

Diamond, B.A., Yelton, D.E. and Scharff, M.D. (1981). Monoclonal antibodies. A new technology for producing serologic reagents. New Eng. J. Med. 304: 1344-1349.

Dixon, F.J. and Maurer, P.H. (1955). Immunologic unresponsiveness induced by protein antigens. J. Exp. Med. 101: 245-257.

Doherty, P.C. and Zinkernagel, R.M. (1975). A biological role for the major histocompatibility antigens. Lancet, 1: 1406-1409.

Donohoe, J.A., Andrus, L., Bowen, K.M., Simeonovic, C., Prowse, S.J. and Lafferty, K.J. (1983). Cultured thyroid allografts induce a state of partial tolerance in adult recipient mice. Transplantation, 35: 62-67.

Doria, G., Agarossi, G. and Adorini, L. (1982). Selective effects of ionizing radiations on immunoregulatory cells. Immunol. Rev. 65: 23-54.

Dorsch, S. and Roser, B. (1974). The clonal nature of allo-antigen-sensitive small lymphocytes in the recirculating pool of normal rats. Aust. J. Exp. Biol.Med. Sci. 52: 45-66.

Dorsch, S. and Roser, B. (1975). T cells mediate transplantation tolerance. Nature, 258: 233-235.

Dorsch, S. and Roser, B. (1977). Recirculating, suppressor T cells in transplantation tolerance. J. Exp. Med. 145: 1144-1157.

Dorsch, S. and Roser, B. (1982a). Suppressor cells in transplantation tolerance. I. Analysis of the suppressor status of neonatally and adoptively tolerized rats. Transplantation, 33: 518-524.

Dorsch, S. and Roser, B. (1982b). Suppressor cells in transplantation tolerance. II Identification and probable mode of action of chimeric suppressor cells. Transplantation, 33: 525-529.

Droege, W. and Mayor, K. (1975). Graft-versus-host reactivity and inhibitory serum factors in allograft-tolerant chickens. Transplantation, 19: 517-520.

Dubernard, J.M., Carpenter, C.B., Busch, G.J.. Diethelm, A.G. and Murray, J.E. (1968). Rejection of canine renal allografts by passive transfer of sensitized serum. Surgery, 64: 752-760.

Duc, H.T., Kinsky, R.G. and Voisin, G.A. (1982). Passive allograft enhancement by subclasses of polyclonal antibodies directed towards restricted regions of the major histocompatibility complex. Transplantation, 33: 492-499.

Dunsford, I., Bowley, C.C., Hutchinson, A.M., Thompson, J.S., Sanger, R. and Race, R.R. (1953). Human blood group chimera. Brit. Med. J. 2: 81.

Du Toit, D.F., Reece-Smith, H., McShane, P., Denton, T., and Morris, P.J. (1982). The effect of Cyclosporin A on allotransplanted pancreatic fragments to the spleen of totally pancreatectomized dogs. Transplantation, 33: 302-307.

Ebringer, A. (1981). The problem of self-tolerance in F_1 animals. Immunology Today, 2: 103-104.

Ehrlich, P. (1908). Uber den jetzigen stand der karzinomparschung. In: The Collected Papers of Paul Erhlich, Vol. 2, Immunology and Cancer Research. Himmelweit, F. (ed). Pergamon Press, London (1957).

Elkins, W.L. and Guttmann, R.D. (1968). Pathogenesis of a local graft versus host reaction: Immunogenicity of circulating host leukocytes. Science, 159: 1250-1251.

Elkins, W.L. (1971). The sources of immunogenic stimulation of lymphoid cells mediating a local graft versus host reaction in rat kidney. Transplantation, 11: 551-560.

Elkins, W.L. (1973). The cellular basis of transplantation tolerance. Transplant. Proc. 5: 685-689.

Elkins, W.L. (1979). Tolerance and the fate of allogeneic bone marrow chimaera. In: Immunological Tolerance and Enhancement. Stuart, F.P. and Fitch, F.W. (eds). MTP Press Ltd., Lancaster. pp. 1-28.

Engers, H.D., Sorenson, G.D., Terres, G., Horvath, C. and Brunner, K.T. (1982). Functional activity in vivo of effector T cell populations. I. Anti-tumour activity exhibited by allogeneic mixed leukocyte culture cells. J. Immunol. 129: 1292-1298.

Fabre, J.W. and Morris, P.J. (1972). The mechanism of specific immunosuppression of renal allograft rejection by donor strain blood. Transplantation, 14: 634-640.

Fabre, J.W. and Morris, P.J. (1973). Dose response studies in passive enhancement of rat renal allografts. Transplantation, 15: 397-403.

Fabre, J.W. and Morris, P.J. (1974). Passive enhancement of homozygous renal allografts in the rat. Transplantation 18: 429-435.

Fabre, J.W. and Morris, P.J. (1975). Studies on the specific suppression of renal allograft rejection in presensitized rats. Theoretical and clinical implications. Transplantation 19: 121-133.

Fabre, J.W. and Batchelor, J.R. (1975). Passive enhancement of renal allografts. Specificity of the enhancing antisera. Transplantation, 20: 269-271.

Fabre, J.W. (1982). Rat kidney allograft model: was it all too good to be true? Transplantation, 34: 223-225.

Fassbinder, W., Frei, U., Persijn, G., Bechstein, P.B., Schopow, K., Dathe, G., Jonas, D., Webber, W., Kuehnl, P. and Schoeppe, W. (1982). Graft survival in renal allograft recipients transfused perioperatively only. Transplant. Proc. 14: 164-167.

Faustman, D., Haupfeld, V., Davie, J.M., Lacy, P.E. and Shreffler, D.C. (1980). Murine pancreatic B cells express H-2K and H-2D but not Ia antigens. J. Exp. Med. 151: 1563-1568.

Faustman, D., Haupfeld, V., Lacy, P. and Davie, J. (1981). Prolongation of murine islets allograft survival by pretreatment of islets with antibody directed to Ia determinants. Proc. Natl. Acad. Sci. (USA) 78: 5156-5159.

Faustman, D., Haupfeld, V., Lacy, P. and Davie, J. (1982a). Demonstration of active tolerance in maintenance of established islets of Langerhans allografts. Proc. Natl. Acad. Sci. (USA) 79: 4153-4155.

Faustman, D., Lacy, P., Davie, J. and Hauptfeld, V. (1982b). Prevention of allograft rejection by immunization with donor blood depleted of Ia bearing cells. Science, 217: 157-158.

Faustman, D., Kraus, P., Lacy, P.E., Finke, E.H. and Davie, J.M. (1982c). Survival of heart allografts in non-immunosuppressed murine recipients by pretreatment of the donor tissue with anti-Ia antibodies. Transplantation, 34: 302-305.

Feduska, N.J., Amend, W.J., Vincenti, F., Perkins, H.A. Opelz, G., Terasaki, P.I., Iwaki, Y., Duca, R., Dodge, D. and Salvatierra, O. (1981). Graft survival with high level of cytotoxic antibodies. Transplant. Proc. 13: 73-80.

Fefer, A. and Nossal, G.J.V. (1962). Abolition of neonatally-induced homograft tolerance in mice by sublethal x-irradiation. Transplant. Bull. 29: 73-79.

Fehrman, I., Groth, C.G., Lundgren, G., Moller, E. (1980). Improved renal graft survival in transfused uremics. A study of a number of interacting factors. Transplantation, 30: 324-327.

Fehrman, I., Groth, C.G., Lundgren, G., Magnusson, G., Ringden, O. and Moller, E. (1982). Effect of blood transfusions on immune response and kidney graft survival. Transplant. Proc. 14: 156-159.

Feldman, J.D. (1972). Immunological enhancement: A study of blocking antibodies. Adv. Immunol. 15: 167-214.

Fell, H.B. (1976). The development of organ culture In: Organ Culture and Biomedical Research. Balls, M. and Monnickendam, M.A. (eds). Cambridge University Press, Cambridge, pp. 1-13.

Felton, L.D. (1949). The significance of antigen in animal tissues. J. Immunol. 61: 107-117.

Felton, L.D., Kauffmann, G., Prescott, B. and Ottinger, B. (1955). Studies on the mechanism of the immunological paralysis induced in mice by pneumococcal polysaccharides. J. Immunol. 74: 17-26.

Finch, D.R.A. and Morris, P.J. (1976). Passive enhancement of isolated pancreatic islet allografts. Transplantation 22: 508-512.

Fine, R.N., Batchelor, J.R., French, M.E. and Shumak, K.H. (1973). The uptake of ^{125}I-labelled rat alloantibody and its loss after combination with antigen. Transplantation, 16: 641-648.

Floersheim, G.L. (1969). A study of combined treatment with chemical immunosuppressants and antilymphocyte serum to prolong skin allograft survival. Transplantation 8: 392-402.

Floerscheim, G.L. (1973). Induction of unresponsiveness to skin and heart allografts in mice by a synergistic treatment with procarbazine antilymphocyte serum, and donor-type cells. Transplantation, 15: 195-205.

Fox, I.J., Perry, L.L., Sy, M.S., Benacerraf, B. and Greene, M.I. (1980). The influence of ultraviolet light irradiation on the immune system. Clin. Immunol. Immunopath. 17: 141-155.

Fox, I.J., Sy, M.S., Benacerraf, B. and Greene, M.I. (1981). Impairment of antigen-presenting cell function by ultraviolet radiation. Transplantation 31: 262-265.

Frangipane, L.G., Poole, T.W., Barker, C.F. and Silvers, W.K. (1977a). Vulnerability of allogeneic xenogeneic pancreatic islets to alloantisera. Transplant. Proc. 9: 371-373.

Frangipane, L.G., Barker, C.F. and Silvers, W.K. (1977b). Importance of weak histocompatibility factors in survival of pancreatic islet transplants. Surg. Forum. 28: 294-296.

French, M.E. and Batchelor, J.R. (1969). Immunological enhancement of rat kidney grafts. Lancet, 2: 1103-1106.

French, M.E., Batchelor, J.R. and Watts, H.G. (1971). The capacity of lymphocytes from rats bearing enhanced kidney allografts to mount graft-versus-host reactions. Transplantation 12: 45-48.

French, M.E. (1972). The early effects of alloantibody and complement on rat kidney allografts. Transplantation 13: 447-451.

French, M.E., and Batchelor, J.R. (1972). Enhancement of renal allografts in rats and man. Transplant. Rev. 13: 115-141.

Gaillard, P.J. (1948). Growth, differentiation and function of explants of some endocrine glands. Symp. Soc. Exp. Biol. Growth, 2: 139-144.

Gaillard, P.J. (1953). Growth and differentiation of explanted tissues. Inter. Rev. Cytology. 2: 331-401.

Gaillard, P.J. (1954). Transplantation of cultivated parathyroid gland tissue in man. In: Ciba Foundation Symposium, Preservation and transplantation of normal tissue. Wolstenholme, G.E.W. and Cameron, M.P. (eds). J and A Churchill Ltd., London.

Gallico, G.G., Butcher, G.W. and Howard, J.C. (1979). The role of subregions of the rat major histocompatibility complex in the rejection and passive enhancement of renal allografts. J. Exp. Med. 149: 244-253.

Gengozian, N., Batson, J.S., Greene, C.T. and Gosslee, D.G. (1969). Hemopoietic chimerism in imported and laboratory-bred marmozets. Transplantation, 8: 633-652.

Gerlag, P.G.G., Koene, R.A., Hagemann, J.F. and Wijdeveld, P.G. (1975). Hyperacute rejection of skin allografts in the mouse. Sensitivity of ingrowing skin grafts to the action of alloantibody and rabbit complement. Transplantation 20: 308-313.

Gerlag, P.G., Capel, P.J., Hagemann, J.F. and Koene, R.A. (1980). Adaptation of skin grafts in the mouse to antibody-mediated rejection. J. Immunol. 125: 583-586.

Gorczynski, R.M. and Macrae, S. (1979). Suppression of cytotoxic response to histoincompatible cells. I. Evidence for two types of T lymphocyte-derived suppressors acting at different stages in the induction of a cytotoxic response. J. Immunol. 122: 737-746.

Gorer, P.A. (1937). The genetic and antigenic basis of tumour transplantation. J. Pathol. Bacteriol. 44: 691-697.

Gose, J.E. and Bach, F.H. (1979). H-2 I-region encoded targets in allograft rejection. J. Exp. Med. 149: 1254-1259.

Gowans, J.L., McGregor, D.D. and Cowen, D.M. (1963). The role of small lymphocytes in the rejection of homografts of skin. In: The Immunologically Competent Cell. CIBA Foundation Study Group No. 16. Wolstenholme, G.E. and Knight, J. (eds). J.A. Churchill Ltd., London.

Graff, R.J. (1978). Minor histocompatibility genes and their antigens. In: Origins of Inbred Mice. Morse, H.C. (ed). Academic Press, New York, pp. 371-389.

Green, C.F. (1982). Cyclosporin-A Therapeutic Promise Maintained. Immunology Today, 3: 121-123.

Greene, M.I., Sy, M.S., Kripke, M. and Benacerraf, B. (1979). Impairment of antigen-presenting cell function by ultraviolet irradiation. Proc. Natl. Acad. Sci. USA 76: 6591-6599.

Greineder, D.K. and Rosenthal, A.S. (1975). Macrophage activation of allogeneic lymphocyte proliferation in the guinea pig mixed leukocyte culture. J. Immunol. 114: 1541-1547.

Gruchalla, R.S. and Streilein, J.W. (1981). Transplantation tolerance: Clonal deletion, active suppression or both? Transplant. Proc. 13: 574-577.

Gruchalla, R.S. and Streilein, J.W. (1982). Analysis of neonatally induced tolerance of H-2 alloantigens. II Failure to detect alloantigen-specific T-lymphocyte precursors and suppressors. Immunogenetics, 15: 111-127.

Guttmann, R.D., Carpenter, C.B., Lindquist, R.R. and Merrill, J.P. (1967). Renal transplantation in the inbred rat, III. A study of heterologous anti-thymocyte sera. J. Exp. Med. 126: 1099-1126.

Guttmann, R.D., Linquist, R.R. and Ockner, S.A. (1969). Renal transplantation in the inbred rat. IX. Hematopoietic origin of an immunogenic stimulus of rejection. Transplantation 8: 472-484.

Guttmann, R.D., Beaudoin, J.G., Morehouse, D.D., Klassen, J., Knaack, J., Jeffrey, J., Chassot, P.G. and Abbou, C.C. (1975). Donor pretreatment as an adjunct to cadaver renal allotransplantation. Transplant. Proc. 7: 117-121.

Halasz, N.A., Orloff, M.J. and Hirose, F. (1964). Increased survival of renal homografts in dogs after injection of graft donor blood. Transplantation 2: 453-458.

Hall, B.M., Dorsch, S. and Roser, B. (1978a). The cellular basis of allograft rejection in vivo. I. The cellular requirements for first-set rejection of heart grafts. J. Exp. Med. 148: 878-889.

Hall, B.M., Dorsch, S. and Roser, B. (1978b). The cellular basis of allograft rejection in vivo. II. The nature of memory cells mediating second-set heart graft rejection. J. Exp. Med. 148: 890-902.

Hammerling, G.J. (1976). Tissue distribution of Ia antigens and their expression on lymphocyte sub-populations. Transplant. Rev. 30: 64-82.

Hanan, R. and Oyama, J. (1954). Inhibition of antibody formation in mature rabbits by contact with the antigen at an early age. J. Immunol. 73: 49-53.

Hanks, J.H. and Wallace, R.E. (1949). Relation of oxygen and temperature in the preservation of tissues by refrigeration. Proc. Soc. Exp. Biol. Med. 71: 196-200.

Hardy, D.A. and Ling, N.R. (1969). Effects of some cellular antigens on lymphocytes and the nature of the mixed lymphocyte reaction. Nature, 221: 545-548.

Hardy, M.A., Fawwaz, R.A., Oluwole, S., Todd, G., Nowygrod, R. and Reemtsma, K. (1979). Selective lymphoid irradiation. I. An approach to transplant-ation. Surgery, 86: 194-200.

Hasek, M. (1959). Some problems of induction of trans-plantation tolerance. In: Biological problems of grafting. Albert, F. and Lejeune-Ledant, G. (eds). Blackwell Scientific Pubs. Oxford pp. 48-63.

Hasek, M., Lengerova, A. and Hraba, T. (1961). Trans-plantation immunity and tolerance. Adv. Immunol. 1: 1-66.

Hasek, M., Chutna, J., Sladecek, M., Machackova, M., Rubenic, J. and Matousek, V. (1975). Attempts to compare the effectiveness of blocking factors and enhancing antibodies in vivo and in vitro. Transplantation 20: 95-100.

Hasek, M. and Chutna, J. (1979). Complexity of the state of immunological tolerance. Immunol. Rev. 46: 3-26.

Hart, D.N.J. and Fabre, J.W. (1979). Quantitative studies on the tissue distribution of Ia and SD antigens in the DA and Lewis rat strains. Transplantation 27: 110-119.

Hart, D.N.J., Winearls, C.G. and Fabre, J.W. (1980). Graft adaptation: Studies on possible mechanisms in long-term surviving rat renal allografts. Transplantation 30: 73-80.

Hart, D.N.J. and Fabre, J.W. (1981a). Localisation of MHC antigens in long surviving rat renal allografts: Probable implication of passenger leucocyte in graft adaptation. Transplant. Proc. 13: 95-99.

Hart, D.N.J. and Fabre, J.W. (1981b). Demonstration and characterisation of Ia-positive dendritic cells in the interstitial connective tissues of rat heart and other tissues but not brain. J. Exp. Med. 153: 347-361.

Hayry, P., Vonwillebrand, E. and Soots, A. (1979). In situ effector mechanisms in rat kidney allograft rejection. III. Kinetics of the inflammatory response and generation of donor-directed killer cells. Scand. J. Immunol. 10: 95-108.

Hayry, P., Vonwillebrand, E. and Anderson, L.C. (1980). Expression of HLA-A, B, C and -DR locus antigens on human kidney, endothelial, tubular and glomerular cells. Scand. J. Immunol. 11: 303-310.

Hellstrom, I., Hellstrom, K.E. and Allison, A.C. (1971). Neonatally induced allograft tolerance may be mediated by serum-borne factors. Nature, 230 49-50.

Hellstrom, K.E. and Hellstrom, I. (1974). Lymphocyte-mediated cytotoxicity and blocking serum activity to tumour antigens. Adv. Immunol. 18: 209-277.

Herbertson, B.M. (1973). The morphology of allograft reactions. In: Immunological aspects of transplantation surgery. Calne, R. (ed). Medical and Technical Pubs. Co. Ltd., Lancaster. pp. 4-38.

Hess, A.D., Tutschka, P.J. and Santos, G.W. (1983). Cyclosporine induced tolerance - mechanism of maintaining specific immunologic unresponsiveness. Transplant. Proc. 15: 539-546.

Heuer, J., Bruner, K., Opalka, B. and Kolsch, E. (1982). A cloned T-cell line from a tolerant mouse represents a novel antigen-specific suppressor cell type. Nature 296: 456-459.

Heyworth, M.F. (1982). Clinical experience with anti-lymphocyte serum. Immunol. Rev. 65: 79-97.

Hilgert, I. (1979). The involvement of activated specific suppressor T cells in maintenance of transplantation tolerance. Immunol. Rev. 46: 27-53.

Hirschberg, H., Evensen, S.A. Henriksen, T. and Thorsby, E. (1975). The human mixed lymphocyte-endothelium culture interaction. Transplantation, 19: 495-504.

Hirschberg, H., Moen, T. and Thorsby, E. (1979). Specific destruction of human endothelial cell monolayers by anti-DRw antisera. Transplantation 28: 116-120.

Hirschberg, H. and Thorsby, E. (1981). Immunogenicity of foreign tissues. Transplantation 31: 96-97.

Hodges, G.M. (1976). A review of methodology in organ culture. In: Organ Culture and Biomedical Research, Balls, M. and Monnickendam, M.A. (eds). Cambridge University Press, Cambridge, pp. 15-59.

Hodgkin, P.D., Dixon, J.E., Andrus, L. and Lafferty, K.J. (1983). Cyclosporin A: A tool for analysing the function of class I MHC antigen-reactive T cells in vivo. Transplant. Proc. (In press).

Holan, B., Hilgert, I., Chutna, J. and Hasek, M. (1980). The regulatory role of I-J subregion in neonatal tolerance induction to H-2D alloantigens. J. Immunogenet. 7: 221-228.

Hood, L., Steinmetz, M. and Malissen, B. (1983). Genes of the major histocompatibility complex of the mouse. Ann. Rev. Immunol. 1: 529-568.

Horaguchi, A. and Merrell, R.C. (1981). Preparation of viable islet cells from dogs by a new method. Diabetes, 30: 455-458.

Horsburgh, T., Wood, P.J. and Brent, L. (1981). In vivo induction of specific suppressor lymphocytes to histo-incompatibility antigens. Transplant. Proc. 13: 637-639.

Hraba, T. (1977). General tolerance phenomena. In: Transplantation. Masshoff, J.W. (ed) Springer Verlag, Berlin. pp. 247-274.

Hume, D.M. (1974). Organ transplants and immunity. In: Immunobiology. Good, R.A. and Fisher, D.W. (eds). Sinauer Association Inc. Pub. Sunderland, Mass. pp. 185-194.

Hutchinson, I.V. (1980). Antigen-reactive cell opsonization (ARCO) and its role in antibody-mediated immune suppression. Immunol. Rev. 49: 167-197.

Hutchinson, I.V. and Brent, L. (1981). Immunological enhancement of tumour allografts following treatment of mice with TNP-conjugated alloantigen and anti-TNP antibody. Nature, 292: 353-355.

Hutchinson, I.V., Shadur, C.A., Duarte, A, Baldwin, W.N., Strom, T.B. and Tilney, N.L. (1981). Mechanisms of cardiac allograft prolongation by Cyclosporin A. Transplant. Proc. 13: 412-413.

Jacobs, B.B. and Huseby, R.A. (1967). Growth of tumours in allogeneic hosts following organ culture explantation. Transplantation 5: 410-419.

Jacobs, B.B. and Huseby, R.A. (1968). Successful growth of tumour allografts following explantation: Effect of various culture conditions. Soc. Exp. Biol. Med. Proc. 127: 957-960.

Jacobs, B.B. (1969). Growth of tumors in allogeneic hosts after organ culture explantation. II. Tumor-host interactions. Nat. Cancer Inst. J. 42: 536-543.

Jacobs, B.B. (1974). Ovarian allograft survival. Prolongation after passage in vitro. Transplantation, 18: 454-457.

Janossy, T., Vegh, P., Tabith, K., Erdos, E. and Petri, G. (1983). Suppressor cells in mouse allograft tolerance. Transplant. Proc. 15: 859-861.

Jansen, J.L.J., Koene, R.A.P., Kamp, V.J.G., Hagemann, H.F.M. and Wijdeveld, P.G.A.B. (1975a). Hyperacute rejection and enhancement of mouse skin graft by antibodies with a distinct specificity. J. Immunol. 115: 392-394.

Jansen, J.L.J., Koene, R.A.P., Kamp, V.G.J., Tamboer, W.P.M. and Wijdeveld, P.G.A.B. (1975b). Isolation of pure IgG subclasses from mouse alloantiserum and their activity in enhancement and hyperacute rejection of skin allograft. J. Immunol. 115: 387-391.

Jeekel, J.J., McKenzie, I.F.C. and Winn, H.J. (1972). Immunological enhancement of skin grafts in the mouse. J. Immunol. 108: 1017-1024.

Jondal, M., Holm, G. and Wigzell, H. (1972). Surface markers on human B and T lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. J. Exp. Med. 136: 207-215.

Jooste, S.V., Winn, H.J. and Russel, P.S. (1973). Destruction of rat skin grafts by humoral antibodies. Transplant. Proc. 5: 713-716.

Jooste, S.V. and Winn, H.J. (1975). Acute destruction of rat skin grafts by alloantisera. J. Immunol. 114: 933-938.

Jooste, S.V., Colvin, R.B., Soper, W.D. and Winn, H.J. (1981a). The vascular bed as the primary target in the destruction of skin grafts by antiserum. I. Resistance of freshly placed xenografts of skin to antiserum. J. Exp. Med. 154: 1319-1331.

Jooste, S.V., Colvin, R.B. and Winn, H.J. (1981b). The vascular bed as the primary target in the destruction of skin grafts by antiserum. II. Loss of sensitivity to antiserum in long-term xenografts of skin. J. Exp. Med. 154: 1332-1341.

Kaliss, N. and Molomut, N. (1952). The effect of prior injections of tissue antiserums on the survival of cancer homoiografts in mice. Cancer Res. 12: 110-112.

Kaliss, N. and Bryant, B.F. (1958). Factors determining destruction and immunological enhancement in mice receiving successive tumour inocula. J. Natl. Cancer Inst. 20: 691-704.

Kaplan, H.S. (1981). Selective effects of total lymphoid irradiation (TLI) on the immune response. Transplant. Proc. 13: 425-428.

Kasahara, K., White. D.J.G., and Calne, R.Y. (1982). Antigen dependence of Cyclosporin A-induced allograft acceptance. Transplantation, 34: 216-218.

Kedinger, M., Haffen, K., Grenier, J. and Eloy, R. (1977). In vitro culture reduces immunogenicity of pancreatic endocrine islets. Nature, 270: 736-738.

Kilshaw, P.J., Brent, L. and Thomas, A.V. (1974). Specific unresponsiveness to skin allografts in mice. II. The mechanism of unresponsiveness induced by tissue extracts and antilymphocytic serum. Transplantation 17: 57-69.

Kilshaw, P.J., Brent, L. and Pinto, M. (1975). Suppressor T cells in mice made unresponsive to skin allografts. Nature, 255: 489-491.

Kindred, B. (1971). Immunological unresponsiveness of genetically thymusless (nude) mice. Eur. J. Immunol. 7: 59-61.

Kissmeyer-Nielsen, F., Olsen, S., Petersen, C.P. and Fjeldborg, O. (1966). Hyperacute rejection of kidney allografts, associated with pre-existing humoral anti-bodies against donor cells. Lancet, 2: 662-665.

Klein, J. (1975). 'Biology of the mouse histocompatibility-2 Complex'. Springer-Verlag, Berlin, New York.

Klein, J., Flaherty, L., Vandeberg, J.L. and Shreffler, D.C. (1978). H-2 haplotypes, genes, regions and antigens: First listing. Immunogenet. 6: 489-512.

Klein, J. (1979). The major histocompatibility complex of the mouse. Science, 203: 516-521.

Klein, J. (1981). Demystifying the major histocompatibility complex. A call for rational nomenclature. Immunology Today, 2: 166-168.

Klein, J., Juretic, A., Baxevanis, C.N. and Nagy, Z.A. (1981). The traditional and a new version of the mouse H-2 complex. Nature, 291: 455-460.

Koene, R.A. and McKenzie, I.F. (1973). A comparison of the cytolytic action of guinea pig and rabbit complement on sensitized nucleated mouse cells. J. Immunol. 111: 1894-1901.

Koene, R.A., Gerlag, P.G., Hageman, J.F., Van Haelst, U.J. and Wijdeveld, P.G. (1973). Hyperacute rejection of skin allografts in the mouse by the administration of alloantibody and rabbit complement. J. Immunol. 111: 520-526.

Kretschmer, R.R. and Perez-Tamayo, R. (1961). The role of humoral antibodies in rejection of skin homografts in rabbits. I. Passive transfer of isoimmune serum to conditioned hosts. J. Exp. Med. 114: 509-519.

Lacy, P.E. and Kostianovsky, M. (1967). Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes, 16: 35-39.

Lacy, P.E., Davie, J.M., Finke, E.H. and Scharp, D.W. (1979a). Prolongation of islet allograft survival. Transplantation, 27: 171-174.

Lacy, P.E., Davie, J.M. and Finke, E.H. (1979b). Prolongation of islet allograft survival following in vitro culture (24°C) and a single injection of ALS. Science, 204: 312-313.

Lacy, P.E., Davie, J.M. and Finke, E.H. (1979c). Induction of rejection of successful allografts of rat islets by donor peritoneal exudate cells. Transplantation, 28: 415-420.

Lacy, P.E., Davie, J.M. and Finke, E.H. (1980a). Effect of culture on islet rejection. Diabetes, 29, Suppl. 1. 93-97.

Lacy, P.E., Davie, J.M. and Finke, E.H. (1980b). Prolongation of islet xenograft survival without continuous immunosuppression. Science, 209: 283-285.

Lacy, P.E., Davie, J.M. and Finke, E.H. (1981). Prolongation of islet xenograft survival (rat to mouse). Diabetes, 30: 285-291.

Lacy, P.E., Finke, E.H., Janney, C.G. and Davie, J.M. (1982). Prolongation of islet xenograft survival by in vitro culture of rat megaislets in 95%O_2. Transplantation, 33: 588-592.

Lafferty, K.J. and Jones, M.A.S. (1969). Reactions of the graft-versus host (GVH) type. Aust. J. Exp. Biol. Med. Sci. 47: 17-54.

Lafferty, K.J., Walker, K.Z., Scollay, R.G. and Killby, B.A.A. (1972). Allogeneic interactions provide evidence for a novel class of immunological reactivity. Transplant. Rev. 12: 198-228.

Lafferty, K.J., Misko, I.S. and Cooley, M.A. (1974). Allogeneic stimulation modulates the in vitro response of T cells to transplantation antigen. Nature, 249: 275-276.

Lafferty, K.J. and Cunningham, A.J. (1975). A new analysis of allogeneic interactions. Aust. J. Exp. Biol. Med. Sci. 53: 27-42.

Lafferty, K.J., Cooley, M.A., Woolnough, J. and Walker, K.Z. (1975). Thyroid allograft immunogenicity is reduced after a period in organ culture. Science, 188: 259-261.

Lafferty, K.J. and Talmage, D.W. (1976). Theory of allogeneic reactivity and its relevance to transplantation biology. Transplant. Proc. 8: 349-353.

Lafferty, K.J., Bootes, A., Dart, G. and Talmage, D.W. (1976a). Effect of organ culture on the survival of thyroid allografts in mice. Transplantation, 22: 138-149.

Lafferty, K.J., Bootes, A., Killby, V.A.A. and Burch, W. (1976b). Mechanism of thyroid allograft rejection. Aust. J. Exp. Biol. Med. Sci. 54: 573-586.

Lafferty, K.J. and Woolnough, J. (1977). The origin and mechanism of the allograft reaction. Immunol. Rev. 35: 231-262.

Lafferty, K.J., Warren, H.S., Woolnough, J.A. and Talmage, D.W. (1978). Immunological induction of T lymphocytes: Role of antigen and the lymphocyte costimulator. Blood Cells, 4: 395-404.

Lafferty, K.J. (1980). Immunogenicity of foreign tissues. Transplantation 29: 179-182.

Lafferty, K.J., Andrus, L. and Prowse, S.J. (1980). Role of lymphokine and antigen in the control of specific T cell responses. Immunol. Rev. 51: 279-314.

Lafferty, K.J., Prowse, S.J., Simeonovic, C.J. and Warren, H.S. (1983a). Immunobiology of tissue transplantation: A return to the passenger leucocyte concept. Ann. Rev. Immunol. 1: 143-173.

Lafferty, K.J., Borel, J.F. and Hodgkin, P. (1983b). Cyclosporine A (CsA): Models for the mechanisms of action. Transplant. Proc.(In Press).

Lance, E.M., Medawar, P.B. and Taub, R.N. (1973). Anti-lymphocyte serum. Adv. Immunol. 17: 1-92.

Larsson, E-L., Coutinho, A. and Martinez, A.C. (1980). A suggested mechanism for T lymphocyte activation: Implications on the acquisition of functional reactivities. Immunol. Rev. 51: 61-91.

Lau, H., Reemtsma, K. and Hardy, M.A. (1983). Pancreatic islet allograft prolongation by donor-specific blood transfusion treated with ultraviolet irradiation. Science, 221: 754-756.

Law, L.W., Appella, E., Cohen, J.M. and Wright, P.W. (1974). Role of serum factors from tolerant mice in blocking and in immunological enhancement. Transplantation, 18: 14-19.

Lechler, R.I. and Batchelor, J.R. (1982). Restoration of immunogenicity to passenger cell-depleted kidney allografts by the addition of donor strain dendritic cells. J. Exp. Med. 155: 31-41.

Ledbetter, J.A., Rouse, R.V., Micklem, H.S., Herzenberg, L.A. (1980). T cell subsets defined by expression of Lyt-1, 2,3 and Thy-1 antigens. 2-parameter immunofluorescence and cytotoxicity analysis with monoclonal antibodies modifies current views. J. Exp. Med. 152: 280-295.

Lenhard, V., Seifert, P., Maassen, G. Grosse-Wilde, H., Johannsen, R., Geisen, H.P. and Kluge, A. (1982). Blood transfusion-induced suppression of cellular immunity in prospective kidney-graft recipients. Transplant. Proc. 14: 160-163.

Lernmark, A. and Baekkeskov, S. (1981). Islet cell antibodies - Theoretical and practical implications. Diabetologia, 21: 431-435.

Leung, K.N. and Ada, G.L. (1980). Two T-cell populations mediate delayed type hypersensitivity to murine influenza virus infection. Scand. J. Immunol. 12: 481-487.

Lindahl-Kiessling, K. and Safwenberg, J. (1971). Inability of UV-irradiated lymphocytes to stimulate allogeneic cells in mixed lymphocyte culture. Int. Arch. Allergy. 41: 670-678.

Lindquist, R.R., Guttmann, R.D. and Merrill, J.P. (1971). Renal transplantation in the inbred rat. VI. Electronmicroscopic study of the mononuclear cells accummulating in rejecting renal allografts. Transplantation, 12: 1-10.

Loeb, L. (1930). Transplantation and individuality. Physiol. Reviews, 10: 547-616.

Loeb, L. (1945). 'The biological basis of individuality'. Charles C. Thomas, Springfield, Illinois.

Lord, E.M. and Nardella, G. (1980). The multicellular tumor spheroid model. Transplantation, 29: 119-124.

Lotze, M.T., Line, B.R., Mathisen, D.J. and Rosenberg, S.A. (1980). The in vivo distribution of autologous human and murine lymphoid cells grown in T cell growth factor (TCGF): Implications for the adoptive immunotherapy of tumors. J. Immunol. 125: 1487-1493.

Loveland, B.E., Hogarth, P.M., Ceredig, R.H. and McKenzie, I.F. (1981). Cells mediating graft rejection in the mouse. I. Lyt-1 cells mediate skin graft rejection. J. Exp. Med. 153: 1044-1057.

Loveland, B.E. and McKenzie, I.F. (1982a). Cells mediating graft rejection in the mouse. II. The Ly phenotypes of cells producing tumor allograft rejection. Transplantation, 33: 174-180.

Loveland, B.E. and McKenzie, I.F. (1982b). Which T cells cause graft rejection? Transplantation, 33: 217-221.

Loveland, B.E. and McKenzie, I.F. (1982c). Cells mediating graft rejection in the mouse. IV. Thy Ly-5,6 and 7 effector cell phenotype. Transplantation 33: 411-413.

Lueker, D.C. and Sharpton, T.R. (1974). Survival of ovarian allografts following maintenance in organ culture. Transplantation, 18: 457-458.

Maki, T., Okazaki, H., Wood, M.L. and Monaco, A.P. (1981). Suppressor cells in mice bearing intact skin allografts after blood transfusions. Transplantation 32: 463-466.

Mandel, T.E. and Higginbotham, L. (1979). Organ culture and transplantation of fetal mouse pancreatic islets. Transplant. Proc. 11: 1505-1506.

Manning, D.D., Reed, M.D. and Shaffer, C.F. (1973). Maintenance of skin xenografts of widely divergent phylogenetic origin on congenitally athymic (nude) mice, J. Exp. Med. 138; 488-494.

Marquet, R.L., Heystek, G.A. and Tinbergen, W.J. (1971). Specific inhibition of organ allograft rejection by donor blood. Transplant. Proc. 3: 708-710.

Marquet, R.L. and Heystek, G.A. (1975). The effect of immunosuppressive treatment on the survival of allogeneic islets of Langerhans in rats. Transplantation, 20: 428-431.

Marquet, R.L. and Heystek, G.A. (1981). Induction of suppressor cells by donor-specific blood transfusions and heart transplantation in rats. Transplantation, 31: 272-274.

Maumenee, A.E. (1951). The influence of donor-recipient sensitization on corneal grafts. Amer. J. Opthalmol. 34: 142-152.

McDowall, R.A.W., Batchelor, J.R. and French, M.E. (1973). Hyperacute rejection and enhancement of rabbit-kidney allografts. Lancet 1, 797-801.

McKenzie, I.F.C., Koene, R.A.P. and Winn, H.J. (1972). Evidence for adaptation of skin grafts in enhanced irradiated mice. Transplantation, 14: 661-663.

McKenzie, I.F.C. and Henning, M.M. (1977). The I-region transplantation antigens: Immunogenicity and enhancement. J. Immunogenet. 4: 259-269.

McKenzie, I.F.C. and Henning, M.M. (1978). The differential destructive and enhancing effects of anti-H-2K, H-2D, and anti-Ia antisera on murine skin allografts. J. Exp. Med. 147: 611-616.

McKenzie, I.F. (1983). Alloaggression. Transplant. Proc. 15: 269-273.

MacLennan, I.C.M. (1972). Antibody in the induction and inhibition of lymphocyte cytotoxicity. Transplant. Rev. 13: 67-90.

Medawar, P.B. (1944). The behaviour and fate of skin autografts and skin homografts in rabbits. J. Anat. 78: 176-199.

Medawar, P.B. (1946). Immunity to homologous grafted skin. II. The relationship betwen the antigens of blood and skin. Brit. J. Exp. Path. 27: 15-24.

Medawar, P.B. (1956-1957). The immunology of transplantation. Harvey Lect. 52: 144-176.

Medawar, P.B. (1957). 'The Uniqueness of the Individual'. Methuen and Co. Ltd., London.

Medawar, P.B. and Woodruff, M.F.A. (1958). The induction of tolerance by skin homografts on newborn rats. Immunol. 1: 27-35.

Medawar, P.B. (1963). Introduction: Definition of the immunologically competent cell. In: The immunologically competent cell: Its nature and origin. CIBA Foundation Study Group No. 16, Wolstenholme, G.E.W. and Knight, J. (eds), Churchill, London. pp. 1-3.

Medawar, P.B. (1965). Transplantation of tissues and organs: Introduction. Brit. Med. Bull. 21: 97-99.

Milgrom, F. (1977). Role of humoral antibodies in transplantation. Transplant. Proc. 9: 721-727.

Miller, J.F. (1961). Immunolgical function of the thymus. Lancet 2, 748-749.

Miller, J., Lifton, J. and Hattler, B. (1975). Alteration of the antigenicity of human lymphocytes by plant lectins and short-term tissue culture. Cell Immunol. 19: 306-317.

Millstein, C. (1981). The Welcome Foundation Lecture, 1980. Monoclonal antibodies from hybrid myelomas. Proc. Roy. Soc. Lond. B. 211: 393-412.

Mitchison, N.A. (1953). Passive transfer of transplantation immunity. Nature, 171: 267-268.

Mitchison, N.A. (1954). Passive transfer of transplantation immunity. Proc. Roy. Soc. Lond. B. 142: 72-87.

Mitchison, N.A. (1955). Studies on the immunological response to foreign tumor transplants in the mouse. I. The role of lymph node cells in conferring immunity by adoptive transfer. J. Exp. Med. 102: 157-177.

Mitchison, N.A. and Dube, O.L. (1955). Studies on the immunological response to foreign tumor transplants in the mouse. II. The relation between hemagluttinating antibody and graft resistance in the normal mouse and mice pretreated with tissue preparations. J. Exp. Med. 102: 179-197.

Mitchison, N.A. (1980). The major histocompatibility complex: The modern synthesis. Immunology Today, 1: 91-92.

Mitchison, N.A. (1981). Demystifying the major histocompatibility complex. The case for a functional nomenclature. Immunology Today 2: 167.

Miyajima, T., Higuchi, R., Kashinabara, H., Yokoyama, T. and Fujimato, S. (1980). Anti-idiotypic antibodies in a patient with a functional renal graft. Nature, 283: 306-308.

Möller, G. (1963). Studies on the mechanism of immunological enhancement of tumor homografts. II. Effect of isoantibodies on various tumor cells. Natl. Cancer Inst. J. 30: 1177-1203.

Monaco, A.P., Gozzo, J.J., Wood, M.L. and Liegeois, A. (1971). Use of low doses of homozygous allogeneic bone marrow cells to induce tolerance with anti-lymphocyte serum (ALS): Tolerance by intra-organ injection. Transplant. Proc. 3: 680-683.

Moran, T.M., Dittmer, J., Hartnett, L., Schwadron, R. and Bennett, M. (1983). Immunological studies of transplantation tolerance in irradiated rats repopulated with syngeneic hemopoietic cells. Transplantation, 35: 601-606.

Morris, R. and Lucas, Z.J. (1971). Immunological enhancement of rat kidney grafts: Evidence for peripheral action of homologous antiserum. Transplant. Proc. 3: 697-700.

Morris, P.J. (1980). Suppression of rejection of organ allografts by alloantibody. Immunol. Rev. 49: 93-125.

Moskalewski, S. (1965). Isolation and culture of the islets of Langerhans of the guinea-pig. Gen. Comp. Endocrinol. 5: 342-353.

Mullen, Y. and Hildermann, W.H. (1971). Kidney transplantation genetics and enhancement in rats. Transplant. Proc. 3: 669-672.

Mullen, Y., Takasugi, M. and Hildermann, W.H. (1973). The immunological status of rats with long-surviving (enhanced) kidney allografts. Transplantation, 15: 238-246.

Mullen, Y., Raison, R. and Hildermann, W. (1977). Cytotoxic versus immunoblocking effects of specific alloantibodies. Effects of IgM, IgG and IgG_2 on rat kidney allograft survival. Transplantation, 24: 99-105.

Mullen, Y. (1980). Specific immunosuppresion for fetal pancreas allografts in rats. Diabetes, 29: Suppl. 1, 113-120.

Munroe, A. and Bright, S. (1976). Products of the major histocompatibility complex and their relationship to the immune response. Nature, 264: 145-152.

Myburgh, J.A., Smit, J.A., Browde, S. and Hill, R.R.H. (1980a). Transplantation tolerance in primates following total lymphoid irradiation and allogeneic bone marrow injection. I. Orthotopic liver allografts. Transplantation, 29: 401-404.

Myburgh, J.A., Smit, J.A., Hill, R.R.H. and Browde, S. (1980b). Transplantation tolerance in primates following total lymphoid irradiation and allogeneic bone marrow injection. II. Renal allografts. Transplantation, 29: 405-408.

Myburgh, J.A., Smit, J.A., Browde, S. and Stark, J.H. (1983). Current status of total lymphoid irradiation. Transplant. Proc. 15: 659-667.

Nagao, T., White, D.J.G. and Calne, R.Y. (1982). Kinetics of unresponsiveness induced by a short course of Cyclosporin A. Transplantation, 33: 31-35.

Najarian, J.S. and Foker, J.E. (1972). The expression of immunity: the efferent arc. In: Transplantation, Najarian, J.S. and Simmons, R.L. (eds). Lea and Febiger, Philadelphia. pp. 94-122.

Naji, A., Reckard, C.R., Ziegler, M.M. and Barker, C.F. (1975). Vulnerability of pancreatic islets to immune cells and serum. Surg. 4: 26:459-461.

Naji, A., Barker, C.F. and Silvers, W.K. (1979a). Relative vulnerability of isolated pancreatic islets, parathyroid, and skin allografts to cellular and humoral immunity. Transplant. Proc. 11: 560-562.

Naji, A., Silvers, W.K., Plotkin, S.A., Dafoe, E. and Barker, C.F. (1979b). Successful islet transplantation in spontaneous diabetes. Surg. 86: 218-226.

Naji, A., Silvers, W.K. and Barker, C.F. (1981a). Islet transplantation in spontaneously diabetic rats. Transplant. Proc. 13: 826-828.

Naji, A., Silvers, W.K., Bellgrau, D. and Barker, C.F. (1981b). Spontaneous diabetes in rats: Destruction of islets is prevented by immunological tolerance. Science, 213: 1390-1392.

Nakayama, E. (1982). Blocking of effector cells cytotoxicity and T-cell proliferation by Lyt antisera. Immunol. Rev. 68: 117-134.

Nash, J.R., Peters, M. and Bell, P.R.F. (1977). Comparative survival of pancreatic islets, heart, kidney and skin allografts in rats, with and without enhancement. Transplantation, 24: 70-73.

Natali, P.G., Martino de, C., Marcellini, M., Quaranta, V. and Ferrone, S. (1981). Expression of Ia-like antigens on the vasculature of human kidney. Clin. Immunol. Immunopath. 20: 11-20.

Nerup, J. and Lernmark, A. (1981). Autoimmunity in insulin-dependent diabetes mellitus. Amer. J. Med. 70: 135-141.

Ninnemann, J.L. and Good, R.A. (1974). Allogeneic transplantation of organ cultures without immunosuppression. An evaluation using adult mouse skin. Transplantation, 18: 1-5.

Nixon, D.F., Ting, J. P-Y., and Frelinger, J.A. (1982). Ia antigens on non-lymphoid tissues. Their origins and functions. Immunology Today, 3: 339-342.

Nossal, G.J.V. (1983). Cellular mechanisms of immunologic tolerance. Ann. Rev. Immunol. 1: 33-62.

Obertop, H., Bijnen, A.B., Vriesendorp, H.M. and Westbroek, D.L. (1978). Prolongation of renal allograft survival in DLA tissue-typed beagles after third party blood transfusions, and immunosuppressive treatment. Transplantation, 26: 255-259.

Ockner, S.A., Guttmann, R.D. and Lindquist, R.R. (1970). Renal transplantation in the inbred rat, XIV, Mechanism of the modified rejection produced by bone marrow cell pretreatment. Transplantation, 9: 39-48.

Okazaki, H., Maki, T., Wood, M.L. and Monaco, A.P. (1980). Prolongation of skin allograft survival in immunosuppressed mice by multiple transfusions of non-specific blood. Transplantation, 29: 341-343.

Opelz, G., Mickey, M.R. and Terasaki, P.I. (1972). Identification of unresponsive kidney-transplant recipients. Lancet, 1: 868-871.

Opelz, G., Sengar, D.P.S., Mickey, M.R. and Terasaki, P.I. (1973). Effect of blood transfusions on subsequent kidney transplants. Transplant. Proc. 5: 253-259.

Opelz, G. and Terasaki, P.I. (1974). Lymphocyte antigenicity loss with retention of responsiveness. Science, 184: 464-466.

Opelz, G. and Terasaki, P.I. (1980). Dominant effect of transfusions on kidney graft survival. Transplantation, 29: 153-158.

Opelz, G. and Terasaki, P.I. (1981). Importance of preoperative (not peroperative) transfusions for cadaver kidney transplants. Transplantation, 31: 106-108.

Opelz, G., Graver, B. and Terasaki, P. (1981a). Induction of high kidney graft survival rate by multiple transfusion. Lancet, 1: 1223-1225.

Opelz, G., Mickey, M.R. and Terasaki, P.I. (1981b). Blood transfusions and kidney transplants: Remaining controversies. Transplant. Proc. 13: 136-141.

Opelz, G. and Lenhard, V. (1983). Immunological factors influencing renal allograft survival. Ann. Rev. Med. 34: 133-144.

Opelz, G. and Van Rood, J.J. (1983). Mechanisms responsible for the blood transfusion effect. Transplant. Proc. 15: 1520-1521.

Orosz, C.G., Fidelus, R.K., Roopenian, D.C., Widmer, M.B., Ferguson, R.M. and Bach, F.H. (1982). Analysis of cloned T cell function. I. Dissection of cloned T cell proliferative responses using cyclosporin A. J. Immunol. 129: 1865-1868.

Owen, R.D. (1945). Immunogenetic consequences of vascular anastomoses between bovine twins. Science, 102: 400-402.

Pantelouris, E.M. (1968). Absence of thymus in a mouse mutant. Nature, 217: 370-371.

Parr, E.L. and Oei, J.S. (1973). Paraformaldehyde fixation of mouse cells with preservation of antibody-binding by the H-2 locus. Tissue Antigens, 3: 99-107.

Parr, E.L. (1979). The absence of H-2 antigens from mouse pancreatic B-cells demonstrated by immunoferritin labelling. J. Exp. Med. 150: 1-9.

Parr, E.L., Bowen, K.M. and Lafferty, K.J. (1980a). Cellular changes in cultured mouse thyroid glands and islets of Langerhans. Transplantation, 30: 135-141.

Parr, E.L., Lafferty, K.J., Bowen, K.M. and McKenzie, I.F.C. (1980b). H-2 complex and Ia antigens on cells dissociated from mouse thyroid glands and islets of Langerhans. Transplantation, 30: 142-148.

Paul, L.C., Milford, E.L., Paradysz, J.M. and Carpenter, C.B. (1981). Expression of alloantigens on the endothelium of ACI strain kidneys. Transplant. Proc. 13: 1432-1436.

Payne, L.N. and Jaffe, W.P. (1962). Graft-against-host reactions produced in chick embryos with blood from heterologous avian donors. Transplantation Bull. 30: 20-25.

Pedersen, M.C. and Morris, B. (1974). The role of humoral antibody in the rejection of primary renal allografts in sheep. J. Exp. Med. 140: 619-630.

Pennock, J.L., Reitz, B.A., Beiber, C.P., Aziz, S., Oyer, P.E., Strober, S., Hoppe, R., Kaplan, H.S., Stinson, E.B. and Shumway, N.E. (1981). Survival of primates following orthotopic cardiac transplantation treated with total lymphoid irradiation and chemical immune suppression. Transplantation, 32: 467-473.

Persijn, G.G., Van Leewen, A., Parlevliet, J., Cohen, B., Lansbergen, Q., D'Amaro, J., and Van Rood, J.J. (1981). Two major factors influencing kidney graft survival in Eurotransplant: HLA-DR matching and blood transfusion(s). Transplant. Proc. 13: 150-154.

Pinto, M., Brent, L. and Thomas, A.V. (1974). Specific unresponsiveness to skin allografts in mice. III. Synergistic effects of tissue extracts, Bordetella pertussis, and antilymphocyte serum. Transplantation, 17: 477-486.

Prowse, S.J., Lafferty, K.J., Simeonovic, C.J., Agostino, M., Bowen, K.M. and Steele, E.J. (1982a). The reversal of diabetes by pancreatic islet transplantation. Diabetes, 31: Suppl. 4, 30-37.

Prowse, S.J., Steele, E.J. and Lafferty, K.J. (1982b). Islet allografting without immunosuppression: Reversal of insulitis-associated diabetes and a case of spontaneous juvenile-onset diabetes in mice. Aust. J. Exp. Biol. Med. Sci. 60: 619-627.

Raff, M.C. and Wortis, H.H. (1970). Thymus dependence of θ-bearing cells in the peripheral lymphoid tissues of mice. Immunology, 18: 931-942.

Ramseier, H., (1973). Immunization against abolition of transplantation tolerance. Eur. J. Immunol. 3: 156-164.

Reckard, C.R., Stuart, F.P., Clayman, J.L., Buckingham, F. and Schulak, J.A. (1981). Differential susceptibility of segmental and isolated islet allografts of rat pancreas to rejection and enhancement. Transplant. Proc. 13: 819-822.

Reece-Smith, H., Du Toit, D.F., McShane, P. and Morris, P. (1981). Prolonged survival of pancreatic islet allografts transplanted beneath the renal capsule. Transplantation, 31: 305-306.

Reif, A.E. and Allen, J.M. (1963). Specificity of iso-antisera against leukaemic and thymic lymphocytes. Nature, 200, 1332-1333.

Rieger, M. and Hilgert, I. (1977). The involvement of a suppressor mechanism in neonatally induced allograft tolerance in mice. J. Immunogenet. 4: 61-67.

Roberts, P.J. and Hayry, P. (1976). Effector mechanisms in allograft rejection. I. Assembly of "sponge matrix" allografts. Cell. Immunol. 26: 160-167.

Roberts, P.J. and Hayry, P. (1977). Effector mechanisms in allograft rejection. II. Density, electrophoresis, and size of fractionation of allograft-infiltrating cells demonstrating several classes of killer cells. Cell. Immunol. 30: 236-253.

Rosenstein, M., Eberlein, T., Kemeny, M.M., Sugarbaker, P.H. and Rosenberg, S.A. (1981). In vitro growth of murine T cells. VI. Accelerated skin graft rejection caused by adoptively transferred cells expanded in T cell growth factor. J. Immunol. 127: 566-571.

Roser, B. and Dorsch, S. (1979). The cellular basis of transplantation tolerance in the rat. Immunol. Rev. 46: 55-86.

Roser, B. and Dorsch, S. (1980). Suppressor T cells and tolerance to MHC antigens. In: Aspects of Developmental and Comparative Immunology - 1. Solomon, J.B. (ed). Pergamon, Oxford, pp. 431-437.

Roser, B.J., Herbert, J. and Godden, U. (1983). The role of suppressor cells in transplantation. Transplant. Proc. 15: 698-703.

Rouse, B.T. and Wagner, H. (1972). The *in vivo* activity of *in vitro* immunized mouse thymocytes, II. Rejection of skin allografts and graft-vs-host activity. J. Immunol. 109: 1282-1289.

Rowley, D.A., Fitch, F.W., Stuart, F.P., Kohler, H. and Cozenza, H. (1973). Specific suppression of immune responses. Antibody directed against either antigen or receptors for antigen can suppress immunity specifically. Science, 181: 1133-1141.

Sachs, D.H. (1983). Overview of the MHC in mammalian systems. Transplant.Proc. 15: 40-44.

Salaman, J.R. (1983). Steroids and modern immunosuppression. Brit. Med. J. 286: 1373-1375.

Saunders, J.B. de C.M. (1972). A conceptual history of transplantation. In: Transplantation. Najarian, J.S. and Simmons, R.L. (eds). Lea and Febiger, Philadelphia. pp. 3-25.

Scott, D.W. and Amos, D.B. (1978). Tissue transplantation. In: Immunological Diseases, Vol. 1, 3rd edn. Samter, M. (ed). Little, Brown & Co., USA, pp. 341-358.

Scott, H., Brandtzaeg, P., Hirschberg, H., Solheim, B.G. and Thorsby, E. (1981). Vascular and renal distribution of HLA-DR-like antigens. Tissue Antigens, 18: 195-202.

Selawry, H.P., Cohen, H.L. and Mui, M.M. (1981). The effect of donor strain blood and ALS therapy on pancreatic islet allograft survival in the rat. Diabetes, 30: 947-950.

Serie, J.R., Hickey, G.E., Schmitt, R.V. and Hegre, O.D. (1983). Prolongation of culture-isolated neonatal islet xenografts without immunosuppression. Transplantation, 36: 6-11.

Serrou, B. and Rey, A. (1981). Immunopharmacological effects of irradiation. Cancer Immunol. Immunother. 11: 239-242.

Schellekens, P. Th. and Eijsvoogel, V.P. (1970). Lymphocyte transformation in vitro, III. Mechanism of stimulation in the mixed lymphocyte culture. Clin. Exp. Immunol., 7: 229-239.

Sharav, Y., Weinreb, M.M. and Ickowicz, M. (1969). Behaviour and fate of transplanted tooth buds, III. A simultaneous change in host and graft after allogeneic transplantation. Transplantation, 8: 328-332.

Shaw, J., Caplan, B., Paetkau, V., Pilarski, L.M., Delovitch, T.L. and McKenzie, I.F. (1980). Cellular origins of co-stimulator (IL2) and its activity in cytotoxic T lymphocyte responses. J. Immunol. 124: 2231-2239.

Singal, D.P., Joseph, S. and Szewczuk, M.R. (1982). Possible mechanism of the beneficial effect of pretransplant blood transfusions on renal allograft survival in man. Transplant. Proc. 14: 316-318.

Silver, J., Cecka, J.M., McMillan, M. and Hood, L. (1976). Chemical characterization of products of the H-2 complex. Cold Spring Harb. Symp. Quant. Biol. 41: 369-377.

Simeonovic, C.J., Bowen, K.M., Kotlarski, I. and Lafferty, K.J. (1980). Modulation of tissue immunogenicity by organ culture. Comparison of adult islets and fetal pancreas. Transplantation, 30: 174-179.

Simeonovic, C.J. and Lafferty, K.J. (1981). Effect of organ culture on function of transplanted foetal pancreas. Aust. J. Exp. Biol. Med. Sci. 59: 707-712.

Simeonovic, C.J. and Lafferty, K.J. (1982a). The isolation and transplantation of foetal mouse proislets. Aust. J. Exp. Biol. Med. Sci. 60: 383-390.

Simeonovic, C.J. and Lafferty, K.J. (1982b). Immunogenicity of isolated foetal mouse proislets. Aust. J. Exp. Biol. Med. Sci. 60: 391-395.

Simeonovic, C.J., Agostino, M. and Lafferty, K.J. (1983). Control of diabetes: Comparative immunogenicity and function of foetal pancreas and isolated islets. Transplant. Proc. (In press).

Simonsen, M. (1957). The impact on the developing embryo and newborn animal of adult homologous cells. Acta Path. Microbiol. Scand. 40: 480-500.

Simonsen, M. (1962). Graft versus host reaction. The natural history and applicability as tools of research. Prog. Allergy. 6: 349-467.

Slavin, S. and Fuks, Z. (1979). Transplantation tolerance across major histocompatibility barriers after total lymphoid irradiation. Transplantation, 28: 359-361.

Slavin, S., Strober, S, Fuks, Z. and Kaplan, H.S. (1980). Immunosuppression and organ transplantation tolerance using total lymphoid irradiation. Diabetes, 29: Suppl. 1. 121-123.

Smit, J.A., Hill, R.R., Myburgh, J.A. and Browde, S. (1980). Transplantation tolerance in primates after total lymphoid irradiation and allogeneic bone marrow injection, III. Lymphocyte responsiveness and suppressor cell activity. Transplantation, 30: 107-110.

Smit, J.A., Myburgh, J.A., Hill, R.R.H. and Browde, S. (1981). Suppressor cell activity following total lymphoid irradiation (TLI), bone marrow (BM) injection, and kidney transplantation in baboons. Transplant. Proc. 13: 455-457.

Snell, G.D. (1952). Enhancement and inhibition of the growth of tumour homoiotransplants by pretreatment of the hosts with various preparations of normal and tumour tissue. J. Natl. Cancer Inst. 13: 719-729.

Snell, G.D. (1954). The enhancing effect (or actively acquired tolerance) and the histocompatibility-2 locus in the mouse. J. Natl. Cancer Inst. 15: 665-675.

Snell, G.D. (1957). The homograft reaction. Ann. Rev. Microb. 11: 439-459.

Sollinger, H.W. and Bach, F.H. (1976). Collaboration between in vivo responses to LD and SD antigens of major histocompatibility complex. Nature, 259: 487-488.

Sollinger, H.W., Burkholder, P.M., Rasmus, W.R. and Bach, F.H. (1977). Prolonged survival of xenografts after organ culture. Surgery, 1981: 74-79.

Sordat, B., McDonald, H.R. and Lees, R.K. (1980). The multicellularspheroid as a model tumor allograft. III. Morphological and kinetic analysis of spheroid infiltration and destruction. Transplantation, 29: 103-112.

Soulillou, J-P., Carpenter, C.B., D'Apice, A.J.F. and Strom, T.B. (1976). The role of non-classical, FC receptor-associated Ag-B antigens (Ia) in rat allograft enhancement. J. Exp. Med. 143: 405-421.

Staines, N.A., Guy, K. and Cuthbertson, J.L. (1975). Passive enhancement of H-2 incompatible skin allografts and MLR inhibition by cross-reactive Ia antibodies. J. Immunogenet. 2: 317-322.

Steinman, R.M. (1981). Dendritic cells. Transplantation, 31: 151-155.

Steinmuller, D. (1967). Immunization with skin isografts taken from tolerant mice. Science, 158: 127-129.

Steinmuller, D. (1969). Allograft immunity produced with skin isografts from immunologically tolerant mice. Transplant. Proc. 1: 593-596.

Steinmuller, D. and Lofgreen, J.S. (1977). The "Sk" (skin-specific) rejection phenomena as evidence of the natural role of acquired tolerance. Transplant. Proc. 9: 1385-1391.

Steinmuller, D. (1979). Methods for induction of permanent graft survival. Transplant. Proc. 11: 1198-1207.

Steinmuller, D. (1980). Passenger leukocytes and the immunogenicity of skin allografts. J. Invest. Derm. 75: 107-115.

Stetson, C.A. (1963). The role of humoral antibody in the homograft reaction. Adv. Immunol. 3: 97-130.

Stone, H.B., Owings, J.C. and Gey, G.O. (1934). Transplantation of living grafts of thyroid and parathyroid glands. Ann. Surg. 100: 613-628.

Stormont, C., Weir, W.C. and Lane, L.L. (1953). Erythrocyte mosaicism in a pair of sheep twins. Science, 118: 695-696.

Streilein, J.W. (1979). Neonatal tolerance: Towards an immunological definition of self. Immunol. Rev. 46: 125-146.

Strober, S., Slavin, S., Gottlieb, M., Zan-Bar, I., King, D.P., Hoppe, R.T., Fuks, Z., Grumet, F.C. and Kaplan, H.S. (1979). Allograft tolerance after total lymphoid irradiation (TLI). Immunol. Rev. 46: 87-112.

Strom, T.B., Tilney, N.L., Paradysz, J.M., Bancewicz, J. and Carpenter, C.B. (1977). Cellular components of allograft rejection: identity, specificity, and cytotoxic function of cells infiltrating acutely rejecting allografts. J. Immunol. 118: 2020-2026.

Stuart, F.P., Saitoh, T. and Fitch, F.W. (1968). Rejection of renal allografts: specific immunologic suppression. Science, 160: 1463-1465.

Stuart, F.P., Fitch, F.W. and Rowley, D.A. (1970). Specific suppression of renal allograft rejection by treatment with antigen and antibody. Transplant. Proc. 2: 483-488.

Stuart, F.P., Bastien, E., Holter, A., Fitch, F.W. and Elkins, W.L. (1971). Role of passenger leucocytes in the rejection of renal allografts. Transplant. Proc. 3: 461-464.

Stuart, F.P., McKearn, T.J. and Fitch, F.W. (1976). Immunological enhancement of renal allografts by anti-receptor antibody. Surgery, 80: 130-136.

Stuart, F.P., Weiss, A. and Fitch, F.W. (1979). Induction and maintenance of immunological enhancement. In: Immunological Tolerance and Enhancement. Stuart, F.P. and Fitch F.W. (eds), MTP Press Ltd., Lancaster, England.

Stuart, F.P., McKearn, T.J., Weiss, A. and Fitch, F.W. (1980). Suppression of rat renal allograft rejection by antigen and antibody. Immunol. Rev. 49: 127-165.

Summerlin, W.T., Charlton, E. and Karasek, M. (1970). Transplantation of organ cultures of adult human skin. J. Invest. Derm. 55: 310-316.

Summerlin, W.T. (1973). Allogeneic transplantation of organ cultures of adult human skin. Clin. Immunol. Immunopath. 1: 372-384.

Summerlin, W.T., Broutbar, C., Foanes, R.B., Payne, R., Stutman, D., Hayflick, L. and Good, R.A. (1973). Acceptance of phenotypically differing cultured skin in man and mice. Transplant. Proc. 5: 707-710.

Sutherland, D.E.R. (1981a). Pancreas and islet transplantation, I. Experimental Studies. Diabetologia, 20: 161-185.

Sutherland, D.E.R. (1981b). Pancreas and islet transplantation. II. Clinical trials. Diabetologia, 20: 435-450.

Swain, S.L. and Panfili, P.R. (1979). Helper cells activated by allogeneic H-2K or H-2D differences have a Ly phenotype distinct from those responsive to I differences. J. Immunol. 122: 383-391.

Swain, S.L. and Dutton, R.W. (1980). Mouse T-lymphocyte sub-populations: Relationships between function and Lyt antigen phenotype. Immunology Today, 1: 61-65.

Talmage, D.W., Dart, G., Radovich, J. and Lafferty, K.J. (1976). Activation of transplant immunity: effect of donor leukocytes and thyroid allograft rejection. Science, 191: 385-388.

Talmage, D.W., Woolnough, J.A., Hemmingsen, H., Lopez, L. and Lafferty, K.J. (1977). Activation of cytotoxic T cells by non-stimulating tumour cells and spleen cell factor(s). Proc. Natl. Acad. Sci. USA 74: 4610-4614.

Talmage, D.W. and Dart, G.A. (1978). Effect of oxygen pressure during culture on survival of mouse thyroid allograft. Science, 200: 1066-1067.

Talmage, S.W. (1980). Effects of oxygen, temperature, and time of culture on the survival of mouse thyroid and pancreas allografts. Diabetes, 29: Suppl. 1. 105-106.

Theofilopoulos, A.N. (1980). Immune complexes in humoral immune responses: Suppressive and enhancing effects. Immunology Today, 1: 1-3.

Thomson, A.W. (1983). Immunobiology of cyclosporin A - A review. Aust. J. Exp. Biol. Med. Sci. 61: 147-172.

Tilney, N.L. and Bell, P.R.F. (1974). Studies on enhancement of cardiac and renal allografts in the rat. Transplantation, 18: 31-37.

Tilney, N.L., Garovoy, N.R., Busch, G.J., Strom, T.B., Graves, M.J. and Carpenter, C.B. (1979a). Rejected human renal allografts. Recovery and characteristics of infiltrating cells and antibody. Transplantation, 28: 421-426.

Tilney, N.L., Strom, T.B. and Milford, E.L.(1979b). The role of suppression in the immune responses. In: Immunological tolerance and enhancement. Stuart, F.P. and Fitch, F.W. (eds). MTP Press Ltd., Lancaster. pp. 125-148.

Togawa, A., Oppenheim, J.J., Mizel, S.B., Kirkpatrick, C. and Chedid, L. (1978). Transfer factor (TF) and other stimulants activate human macrophages to produce lymphocyte activation factor (LAF). Fed. Proc. 37: 1589.

Triplett, E.L. (1962). On the mechanism of immunologic self-recognition. J. Immunol. 89: 505-510

Tutschka, P.J., Hess, A.D., Beschorner, W.E. and Santos, G.W. (1981a). Suppressor cells in transplantation tolerance. I. Suppressor cells in the mechanism of tolerance in radiation chimeras. Transplantation, 32: 203-209.

Tutschka, P.J., Ki, P.F., Beschorner, W.E., Hess, A.D. and Santos, G.W. (1981b). Suppressor cells in transplantation tolerance. II. Maturation of suppressor cells in the bone marrow chimera. Transplantation, 32: 321-325.

Tutschka, P.J., Hess, A.D., Beschorner, W.E. and Santos, G.W. (1982). Suppressor cells in transplantation tolerance. III. The role of antigen in the maintenance of transplantation tolerance. Transplantation, 33: 510-514.

Tutschka, P.J., Belanger, R., Hess, A.D., Beschorner, W.E. and Santos, G.W. (1983). Suppressor cells in transplantation tolerance to self and non-self. Transplant. Proc. 15: 853-858.

Turk, J.L. and Parker, D. (1982). Effect of cyclosphosphamide on immunological control mechanisms. Immunol. Rev. 65: 99-113.

Vaiman, M., Daburton, F., Reny, J., Villiers, P.A., De Raberolles, C., Lecompte, Y., Mahouy, G. and Fradelizi, D. (1981). Allograft tolerance in pigs after fractionated lymphoid irradiation. I. Skin grafts after partial lateral irradiation and bone marrow cell grafting. Transplantation, 31: 358-364.

Van Es, A.A., Marquet, R.L., Van Rood, J.J. and Balner, H. (1978). Influence of a single blood transfusion on kidney allograft survival on unrelated rhesis monkeys. Transplantation, 26: 325-330.

Van Rood, J.J. (1983). Pre-transplant blood transfusion: Sure! But how and why? Transplant. Proc. 15: 915-916.

Vegh, P., Erdos, E., Janossy, T. and Petri, G. (1980). Studies on the mechanism of transplantation tolerance. I. Do suppressor cells play a role in the induction and maintenance of neonatal transplantation tolerance? Cell. Immunol. 55: 265-271.

Vesole, D.H., Dart, G.A. and Talmage, D.W. (1982). Rejection of stable cultured allografts by active or passive (adoptive) immunization. Proc. Natl. Acad. Sci. USA, 79: 1626-1628.

Voisin, G.A., Kinsky, R., Jansen, F. and Bernard, C. (1969). Biological properties of antibody classes in transplantation immune sera. Transplantation, 8: 618-632.

Von Willebrand, E., Soots, A. and Hayry, P. (1979a). In situ effector mechanisms in rat kidney allograft rejection, I. Characterization of the host cellular infiltrate in rejecting allograft parenchyma. Cell. Immunol. 46: 309-326.

Von Willebrand, E., Soots, A. and Hayry, P. (1970b). In situ mechanisms in rat kidney allograft rejection. II. Heterogeneity of the effector cells in the inflammatory infiltrate vs. that in the spleen of a recipient rat. Cell. Immunol. 46: 327-336.

Wagner, H., Rollinghoff, M. and Nossal, G.J. (1973). T-cell-mediated immune responses induced in vitro: A probe for allograft and tumor immunity. Transplant. Rev. 17: 3-36.

Wagner, H., Hardt, C., Heeg, K., Pfizenmaier, K., Solbach, W., Bartlett, R., Stockinger, H. and Rollinghoff, M. (1980). T-T cell interactions during cytotoxic T lymphocytes (CTL) responses: T cell derived helper factor (Interleukin 2) as a probe to analyse CTL responsiveness and thymic maturation of CTL progenitors. Immunol. Rev. 51: 215-255.

Waksman, B.H. (1980). Lymphokine research: An historical overview. Lymphokine Reports, 1: 1-5.

Warden, G.D., Reemtsma, K. and Steinmuller, D. (1973). The phenomenon of adaptation: Graft or host? Transplant. Proc. 5: 635-639.

Waterfield, J.D., Bick, P.H., Moller, E. and Moller, G. (1981). Experimentally induced transplantation tolerance. I. Dissociation of effector lymphocytes specific for alloantigens by neonatal tolerance induction. Scand. J. Immunol. 13: 211-217.

Watson, J., Mochizuki, D. and Gillis, S. (1980). T-cell growth factors: interleukin 2. Immunology Today, 1: 113-117.

Watson, J.D. (1981). Lymphokines and the induction of immune responses. Transplantation, 31: 313-317.

Weber, R.A., Cannon, J.A. and Longmire, W.P. (1954). Observations on the regrafting of successful homografts in chickens. Ann. Surg. 139: 473-477.

Weigle, W.O. (1961). The immune response of rabbits tolerant to bovine serum albumin to the injection of other heterologous serum albumins. J. Exp. Med. 114: 111-125.

Weigle, W.O. (1973). Immunological unresponsiveness. Adv. Immunol. 16: 61-122.

Weigle, W.O. (1978). Immunological tolerance. In: Immunological Diseases. Vol. 1, 3rd edn. Samter, M. (ed). Little, Brown and Co., U.S.A.

Welsh, K.I., Batchelor, J.R., Maynard, A. and Vergos, H. (1979). Failure of long-surviving, passively enhanced kidney allografts to provide T-dependent alloimmunity. II. Retransplantation of (ASxAug) F_1 kidneys from AS primary recipients into (ASxWF) F_1 secondary hosts. J. Exp. Med. 150: 465-470.

White, D.J.G., Rolles, K., Ottawa, T. and Turell, O. (1980). Cyclosporin-A-induced long-term survival of fully incompatible skin and heart grafts in rats. Transplant. Proc. 12: 261-265.

White, D.J.G. and Calne, R.Y. (1982). The use of Cyclosporin A immunosuppression in organ grafting. Immunol. Rev. 65: 115-131.

Williams, A.F., Galfre, G. and Milstein, C. (1977). Analysis of cell surfaces by xenogeneic myeloma-hybrid antibodies: Differentiation antigens of rat lymphocytes. Cell. 12: 663-673.

Williams, A.F. (1979). Monoclonal antibodies in transplantation research. Transplantation, 27: 152-155.

Williams, G.M., Hume, D.M., Hudson, R.P., Morris, P.J., Kano, K. and Milgrom, F. (1968). "Hyperacute" renal-homograft rejection in man. New Eng. J. Med. 279: 611-618.

Williams, G.M., Krajewski, C.A., Dagher, F.J., Ter Haar, A.M. Roth, J.A. and Santos, G.W. (1971). Host repopulation of endothelium. Transplant. Proc. 3: 869-872.

Williams, K.A., Ting, A., Cullen, P.R. and Morris, P.J. (1979). Transfusions: Their influence on human renal graft survival. Transplant. Proc. 11: 175-178.

Williams, K.A., Ting, A., French, M.E. and Oliver, D. (1980). Peroperative blood-transfusions improved cadaveric renal-allograft survival in non-transfused recipients. Lancet, 1: 1104-1106.

Wilson, D.B., Silvers, W.K. and Billingham, R.E. (1966). Failure to transfer sensitivity to skin homografts by means of 'immune' lymphoid cells in diffusion chambers. Nature, 209: 1359-1360.

Wilson, D.B. and Nowell, P.C. (1970). Quantitative studies on the mixed lymphocyte interaction in rats. IV. Immunologic potentiality of the responding cells. J. Exp. Med. 131: 391-407.

Winearls, C.G., Fabre, J.W., Hart, D.M.J., Millard, P.R. and Morris, P.J. (1980). Passive enhancement and hyperacute rejection of renal allografts in the rat. Transplantation, 29: 462-467.

Winn, H.J., Baldamus, C.A., Jooste, S.V. and Russell, P.S. (1973). Acute destruction by humoral antibody of rat skin grafted to mice. The role of complement and polymorphonuclear leukocytes. J. Exp. Med. 137: 893-910.

Wonigeit, K. and Pichlmayr, R. (1977). Immunosuppression by antibodies. In: Transplantation. Masshoff, J.W. (ed). Springer-Verlag, Berlin and New York. pp. 695-744.

Wood, M.L., Monaco, A.P., Gozzo, J.J. and Liegeois, A. (1971). Use of homozygous allogeneic bone marrow for induction of tolerance with antilymphocyte serum: Dose and timing. Transplant. Proc. 3: 676-679.

Wood, M.L., Gozzo, J.J. and Monaco, A.P. (1972). Use of antilymphocyte serum and bone marrow for production of immunological tolerance and enhancement: Review and recent experiments. Transplant. Proc. 4: 523-529.

Wood, P., Horsburgh, T. and Brent, L. (1981). Specific unresponsiveness to skin allografts in mice. VI. Graft survival in mice pretreated with blood. Transplantation, 31: 8-14.

Woodruff, M.F.A. and Woodruff, H.G. (1950). The transplantation of normal tissues: with special reference to auto-and homotransplants of thyroid and spleen in the anterior chamber of the eye, and subcutaneously, in guinea-pigs. Phil. Trans. Roy. Soc. Lond. B. 234: 559-582.

Woodruff, M.F.A. (1952). The transplantation of homologous tissue and its surgical applications. Ann. Roy. Coll. Surg. England, 11: 173-194.

Woodruff, M.F.A. (1954). The "critical" period of homografts. Transplantation Bull. 1: 221.

Woodruff, M.F.A. and Simpson, L.O. (1955). Induction of tolerance to skin homografts in rats by injection of cells from the prospective donor soon after birth. Brit. J. Exp. Path. 36: 494-499.

Woodruff, M.F.A. (1957). Cellular and humoral factors in the immunity to skin homografts: Experiments with a porous membrane. Ann. NY Acad. Sci. 64: 1014-1026.

Woodruff, M.F.A (1960). 'The Transplantation of Tissues and Organs', Charles C. Thomas, Springfield, Illinois.

Woodruff, M.F.A. (1959). Evidence of adaptation in homografts of normal tissue. In: Biological problems of grafting. Albert F. and Lejeune-Ledant, G. (eds). Blackwell Scientific Pubs., Oxford, pp. 83-94.

Woodruff, M.F.A. and Symes, M.O. (1962). Evidence of loss of tumour-specific antigen on repeatedly transplanting a tumour in the strain of origin. Brit. J. Cancer, 16: 484-488.

Woodruff, M.F.A. and Anderson, N.A. (1963). Effect of lymphocyte depletion by thoracic duct fistula and administration of anti-lymphocytic serum on the survival of skin homografts in rats. Nature, 200: 702.

Woolnough, J.A. and Lafferty, K.J. (1979). Generation of homogeneous populations of alloreactive T cells in vitro. Aust. J. Exp. Biol. Med. Sci. 57: 127-139.

Woolnough, J.A., Misko, O.S. and Lafferty, K.J. (1979). Cytotoxic and proliferative lymphocyte responses to allogeneic and xenogeneic antigens in vitro. Aust. J. Exp. Biol. Med. Sci. 57: 467-477.

Wortis, H.H. (1971). Immunological responses of 'nude' mice. Clin. Exp. Immunol. 8: 305-317.

Yamashita, U. and Shevach, E.M. (1977). The expression of Ia antigens on immunocompetent cells in the guinea-pig. II. Ia antigens on macrophages. J. Immunol. 119: 1584-1588.

Zimmerman, C.E., Busch, G.J., Stuart, F.P. and Wilson, R.E. (1968). Canine renal homografts after pretreatment with subcellular splenic antigens. Surgery, 63: 437-445.

Zinkernagel, R.M. and Doherty, P.C. (1974a). Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semi-allogeneic system. Nature, 248: 701-702.

Zinkernagel, R.M. and Doherty, P.C. (1974b). Immunological surveillance against altered self components by sensitised T lymphocytes in lymphocytic choriomeningitis. Nature, 251: 547-548.

Zitron, I.M., Ono, J., Lacy, P.E. and Davie, J.M. (1981a). The cellular stimuli for the rejection of established islet allografts. Diabetes, 30: 242-246.

Zitron, I.M., Ono, J., Lacy, P.E. and Davie, J.M. (1981b). Active suppression in the maintenance of pancreatic islet allografts. Transplantation 32: 156-158.